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Colleges back from the dead

United States universities and colleges are celebrating their commencement days with relief. The prospects for the next academic year seemed even worse a few months ago. But they are still grim.

In *Tom Sawyer* the hero attends his own funeral, only later to reveal to everyone that he wasn't dead after all. This spring US colleges and universities are in a situation very much like Tom's. For most of the year, the death of American higher education has been lamented by the higher education lobby in Washington, fighting the cuts in student loan finance proposed in President Reagan's budget. Now, however, it looks as though Congress has heard the cries of middle-class parents throughout the country, and will rescue the education monies so that the young people who need government loans to pay tuition bills can go on attending college in the fall. So higher education is alive, after all.

But to be alive is not to be well. A major debate has been going on in the US education community about the changing shape of US higher education including, literally, the ability of some kinds of schools to survive the next decade. The month of June, when most schools' terms end, when final revenue figures are known and re-enrolment data are available, is a good time to take stock. Moreover the blistering fight over government student loans, which has preoccupied university spokesmen, the Administration and the Congress for months, is now winding down. The smoke is clearing. And everyone agrees that US colleges and universities are under unprecedented stress.

The most obvious source of stress is the declining number of 18-year-olds in the US population projected from the early 1980s onwards. As a study by Princeton's president William Bowen said a few years ago, this could presage a general shrinkage in the size of the US university system. Applications figures for enrolment in the fall of 1982 are down a mite (0.9 per cent), but educationists attribute this decline to other factors, such as rising tuition fees, high interest rates on private loans and the uncertainty of government loans. The population factor will show up, they say, next year, when the number of applicants for the fall of 1983 drop off. Eventually, the drop will mean that schools will have to compete harder to get students (or lower their standards). To anticipate this prospect Bowen recommended that the universities should plan to shrink.

A second source of stress is the financial crisis in state governments, due to the taxpayers' revolt that spread to many states after it began some years back in California, and the loss of revenue from traditional federal programmes under the Reagan Administration. Some states, such as Michigan, Missouri and Minnesota, simply cannot meet expenses any longer, are running enormous deficits and so are cutting back funds to their state universities. State university financing has become an annual roller-coaster that would give ulcers to the average modestly comfortable professor in Cambridge, for example. Some state systems have grown, over the years, into large, comprehensive undergraduate and graduate programmes, of high quality and easy access through nominal tuition fees. They fulfill the best ideals of American education. But some states have laws requiring that the budget be balanced every year. So, for most of the year, university administrators do not know how much money will be given by the legislature for the next year, or whether the university will be the target of some last-minute cut.

The easiest response is to conserve fuel, cut down on office staffs and maintenance, defer new building or expensive new computers or other instrumentation, to shave the number of faculty evenly across the board — and to avoid fights. But that

does not maintain quality. To maintain quality a school must cut out the fat (weak departments) or do away with tenure — both of which can lead to embittered fights on campus and also lawsuits. Another course is to raise tuition fees at state schools above the traditional nominal level (freshmen at the University of Southern California next autumn will have to pay \$7,000, for example). But while this raises revenue, it discourages applicants and contradicts the very idea that state schools are meant to embody high quality education for everyone. None of these remedies is pleasant, let alone conducive to an unperturbed environment for study.

Not all state schools are in this same plight. Some, such as Pennsylvania State University, are doing well. But these are schools with strong ties with local industry and a well-understood tradition of service to the state and the community. Applications for next autumn for Pennsylvania State University, for example, are up 10 per cent. Industry finance is already a school tradition, so that door is open. And so long as the cost of private and other state school tuition keeps rising, prospective students shopping for the best deal will come.

The stresses on private schools are variations on those afflicting the state schools. They cost more, so applications for next fall are down at most of them, according to a survey by John Minter Associates for the *Chronicle of Higher Education*. The swing of the marketplace is clearest in the west and south-west, where 67 per cent of private schools report decreases in applications while 71 per cent of the public schools report increases. Hardest hit of the private schools are the mid-ranked colleges, particularly liberal arts colleges, which cannot advertise quickly usable skills to the career-minded young and which must charge substantial tuition fees. The choice facing these schools is either to lower admissions standards or "planned shrinkage".

Meanwhile, the rich get richer. The top private schools are doing well, although they face several shifts in student choices and faculty availability. The top schools often have large and important research laboratories supported by industry and the government, or major graduate schools or medical schools that are sources of overhead to help carry along departments, such as the liberal arts, that do not generally have outside income. Part of the new, conservative public mood in the United States, as Chester E. Finn Jr has written, is a concern with educational quality. And, when parents, students, alumni and industry are all preoccupied with quality, they will tend to gravitate to institutions of proven quality. Thus, Harvard's tuition fees have never been higher (next fall they will be \$8,195) and its fund-raising has never been more successful.

What does all this mean for science? It means that the trends in academic science identified in 1977 by Bruce Smith and William Karlesky* are probably going to be even more pronounced. Smith and Karlesky surveyed many different kinds of US colleges and universities — four-year community colleges, state schools and top rank research institutions. They identified about four principal trends, which would combine, they said, to squeeze out the "middle" of US academic research in the future. At the lower level, the vocational schools would prosper (as, indeed, they have) because when people are unemployed, they go to school to get

*Smith, B. & Karlesky, W. *The State of Academic Science* (Change Magazine Press, Washington D.C., 1977).



skills. But as funds become tight, mid-ranked schools which can compete less well for federal funds, industry gifts and students will become less and less able to carry out a research as well as a teaching role. Their research programmes will diminish. Meanwhile, the top-ranked schools, while facing critical problems such as a decline in places for young faculty, will ride out the storm. Like Tom Sawyer, they will live on.

New ways with weapons

Bilateral negotiations on strategic arms are to begin later in the month. Where will they lead?

The passage of time has curious healing qualities. Two years ago, Mr Ronald Reagan, campaigning successfully for the presidency of the United States, was outspoken in his condemnation of President Jimmy Carter's second instalment of the treaty that emerged from the Strategic Arms Limitations Talks (Salt II), originally devised by Dr Henry Kissinger for once-President Richard M. Nixon. Reagan was pushing at an open door, for his political opponent knew that the US Senate would not ratify the treaty he had signed; indeed, the Soviet occupation of Afghanistan gave him a handy excuse to withdraw his request for ratification without too much loss of face. The Senate's objections to Salt II were largely technical — verification, the equity of the equation of one long-range missile with another and, perhaps more important, the knowledge that nobody from the Carter White House had faced the problem of persuading the Pentagon that the rules of the strategic arms race should be changed. Candidate Reagan could afford to be more radical and rhetorical: how is it possible to trust in an agreement with people whom we do not trust? President Reagan has nevertheless found that his administration has kept within the unratified rules of Salt II, if only because it has been hard to know what should be done about the disposition of the MX missiles, the long-range missiles destined to replace the Minuteman. In the Soviet Union, likewise, the same rules have adventitiously been kept. And now, the emollient passage of time has persuaded President Reagan that he must follow his predecessor's path and propose bilateral talks with the Soviet Union on strategic arms (see *Nature* 20 May, p.169). The surprise is that the Soviet Union has so quickly agreed: the talks are due to begin in Geneva on 27 June.

A modicum of muck-raking is in order even in these hopeful circumstances. The Reagan Administration's original indifference to strategic arms talks appears to have been largely ideological — sup with the devil only with a long spoon, negotiate on strategic arms only from strength. This position has been undermined by predictable (and predicted) forces — political pressure from allied governments, political pressure from domestic voters, fears that another review conference of the Non-Proliferation Treaty (in 1985) as arid as that of 1980 might drive some middle-sized powers into the nuclear club, the sheer cost but also the technical difficulty of developing the new generation of strategic arms and the recognition that the world, with the Falklands and the Middle East, is a dangerous place.

So what should the Reagan Administration be aiming for? Its opening bid is simple enough — let each side be constrained within a limit of 5,000 strategic warheads with the proviso that no more than half of these should be based on land. The proposal is rational enough — submarine missiles are for the time being relatively invulnerable to attack, while ground-based missiles are potentially vulnerable to a first strike by the other side and are thus destabilizing. The Soviet Union's predictable but far from final objection is that the proposal would more immediately affect the Soviet Union than the United States. The success of the Geneva enterprise will crucially depend on the willingness of the two sides to acknowledge that 5,000 nuclear warheads, each of them at least ten times larger than the bombs dropped on Hiroshima and Nagasaki, are outrageously too many for the now classical purposes of deterrence. Indeed, these huge numbers can be explained only if each superpower is prepared to attack the other's ground-based missiles in a supposedly pre-emptive first

strike, in the process presumably inviting an attack on its cities by the other's submarine missiles. Are the two superpowers prepared to return to the simple view that nuclear weapons are intended simply as deterrents?

Other difficulties will arise before that question is faced. How, for example, will the new strategic arms talks be linked with the talks on "theatre nuclear weapons" already under way in Geneva? That the two negotiations should be linked is sensible, while the United States proposal that its FB111 bombers based in Europe should be counted within the separate limit for strategic aircraft is an important concession. But where will the British and even French nuclear forces fit in? And would the attainment of President Reagan's "zero option" for European "theatre weapons" be the equivalent of the nuclear-free zone for which the Palme commission was pressing last week (see page 446)?

These questions are so difficult that there can be no quick end to the proceedings in Geneva. The best hope is that there will be some quick agreement on a warhead ceiling below which further reductions will be sought. During that second stage of the negotiations, the Soviet Union will be faced with the theoretically sensible but politically sensitive proposal by the United States that limits on strategic weapons should be determined not by counting missiles or even warheads but by their throw-weight of carrying capacity. The obvious snag is that this criterion would more severely penalize the Soviet Union (with its large rockets) than the United States, while everybody would know that the effective power of a warhead is at least partly a function of its accuracy, quantifiable but hardly verifiable. This is why the most important potential benefit of the Geneva process is that it may change the way the superpowers think — and that it might in particular persuade people like Mr Alexander Haig, the US Secretary of State, to avoid statements like that the other day in which he said that no strategic arms agreement would be acceptable if it made conventional wars more likely. Is that a sensible criterion? Nobody thinks conventional wars desirable in themselves, but an effective agreement on strategic nuclear weapons must surely be one that will allow conventional warfare to break out without the immediate risk of nuclear conflict.

Waiting for Merrison

British university research is threatened by attrition and neglect. The Merrison report will not help.

After more than two years of brooding, Sir Alec Merrison's committee on the condition of British university research seems to have confirmed people's worst fears but to have failed to show how they may be exorcised. Its report just published (see facing page) is a masterpiece of judiciousness. The dual support system whose collapse in the past decade was the chief reason for the committee's existence is said to be in all ways admirable, and therefore is to be preserved at all costs. Indeed, to judge from what the committee says, there is only one defect in this excellent and peculiarly British device for sharing the cost of university research between one public purse (the University Grants Committee) and another (the research councils): the system appears not to function as intended. In much the same spirit might a man sent to rescue a motor-car fallen in a river report that the car was in excellent condition but that it had sunk.

Nobody, or hardly anybody, is to blame for this debacle. The committee had the bad luck to have been established before the full severity of the British government's intentions towards its universities had become apparent. And the committee's terms of reference (behind which it shelters) were in truth too narrow. It was allowable to observe (as the committee does) that universities' internal arrangements for deciding priorities in their discharge of responsibility for research and scholarship are inadequate, but outside its brief to enquire why things have come to such a pass. The more serious difficulty, however, is that British universities are in such a mess, one so arbitrarily brought about, that the safeguarding of research also requires arbitrariness on somebody's part. Only the research councils are at present capable of being as selective as circumstances require.

Nuclear waste bill now in sight

But critics fear stop-gap stores will last

Washington

Congress is now closer than ever to passing a nuclear waste management bill, but its terms seem likely to be much more appealing to the atomic industry than to environmental groups, both of which, in a strange alliance, have for years been calling for a legislative solution to the growing mass of spent commercial fuel.

The Senate has already passed a bill that the industry is satisfied will end the uncertainty plaguing the government's waste disposal programme. In the absence of legislative directions, each administration has been free to set its own policy, usually inconsistent with that of the previous administration.

The environmental lobby, on the other hand, is worried that the Senate bill and its counterparts now under consideration in the House gloss over the serious technical problems of waste management in favour of political expediency.

At present, 8,000 tonnes of spent commercial fuel is in temporary storage at reactor sites. By the end of the century, the figure is expected to reach 72,000 tonnes. The ultimate solution, everyone seems to agree, is to dispose of it in deep geological repositories. This solution is provided for in all versions of the bill.

The bone of contention, however, has become whether the federal government should in addition provide some form of interim storage. According to the Atomic Industrial Forum, which represents the nuclear power industry, roughly half a dozen reactors will run out of on-site storage space by 1985; the problem will be widespread by the 1990s. The industry is thus very pleased that the Senate bill provides for stop-gap storage to cover any delays in a permanent repository.

The environmental groups see something more sinister going on. The interim storage envisaged in the Senate bill will be of two kinds. The first is "away-from-reactor" (AFR) storage, which is essentially the arrangement used at reactor sites: spent fuel elements are simply stacked in a water-filled "swimming pool" which absorbs the radiation and heat. The environmental groups charge that AFR is a way for the industry to avoid the licensing procedure required to expand on-site storage. The second kind of facility is "monitored retrievable storage" (MRS), which is only vaguely defined. The chief worry among the environmental groups is that MRS will become the *de facto*

permanent solution.

Brooks Yeager of the Sierra Club says that the Senate bill virtually guarantees that. It sets "not just ambitious, but unmeetable deadlines" for the construction of a geological repository. "They want to arrive at the issuance of a construction permit by the end of the decade. That's seven years faster than the Department of Energy's plans for construction in order to resolve all the technical problems." The timetable may also guarantee that the choice of sites will be limited to the three at which the Department of Energy has already begun tests — the Hanford

Reservation in Washington state, the Nevada test site and a group of sites along the Gulf of Mexico.

Professor Henry Kendall of the Massachusetts Institute of Technology, who is active in the Union of Concerned Scientists, a group critical of US nuclear policy, agrees that building an MRS facility "basically means you don't have confidence in permanent disposal". He says that what is needed is time to make a careful hydrological study of the actual site.

These worries seem to be backed up by a recent study by the Congress's Office of Technology Assessment (OTA). The

British research — no cure yet

British university research is in trouble, but only the universities themselves can work their way out of it. This is the chief conclusion of the much delayed report of the committee under Sir Alec Merrison, vice-chancellor of the University of Bristol, set up two and a half years ago to brood about financial support for university research and published this week (Cmnd 8567, HMSO, £4.35).

The report comes down squarely in favour of the British dual-support system, whereby the University Grants Committee (UGC) provides universities with the basic wherewithal for research and the research councils provide extra funds (but no overhead) for particular projects. But the committee also says that "the system has been under strains for several years".

One of the committee's chief proposals is that universities should more deliberately channel part of the funds they receive from the UGC into areas of research in which they consider themselves to be strong, for which purpose it says that British universities should set up research committees to supervise the internal allocation of funds. But the committee also says that as a stop-gap, the research councils should be prepared to "meet costs they would not normally meet" (a euphemism for paying overhead) or think of moving people doing good work in unfavourable circumstances to other places.

The essence of the committee's support for the continuation of the dual-support system is its repeated reaffirmation of the belief that if universities were not provided with funds that can be spent on their own discretion on research projects that would not normally win research council support, genuinely innovative ideas would never see the light of day. It considers but rejects on the same grounds that UGC support for research should be linked directly with the volume of financial support provided by the research councils, while it considers that if UGC were to earmark any but a small proportion of its university support for specific projects, the resulting rigidity

would be self-defeating.

The chief targets for the committee's advice are the universities, which are told that in the long run — "the prospects for achieving any significant shift in the near future are next to impossible" — they must be prepared to spend more of their resources on research rather than teaching, that they should "concentrate research funds into selected areas", look at the problems occasioned by academic tenure, find ways (with the help of the research councils) of bringing in "new blood" and be prepared to form associations with other universities for more effective prosecution of research. Both partners in the dual-support system are asked to be more sensitive to researchers' need to travel.

The research councils are given two principal tasks — to adjust the support provided for graduate students more regularly in tune with the increasing cost of living and to "study" the balance between their support of research in universities and in their own establishments. In a memorable sentence, the report says that "we are not satisfied that the balance of research council expenditure between such support and the work of their own institutes is in all cases right".

The committee's belief that university research is in trouble is based on statistical evidence that the committee says should be improved. The data do, however, show that the decline in research support from universities' own budgets goes back to the early 1970s, and that between the beginning and the end of that decade the average sum of money available to university departments per head of academic staff employed declined by 28 per cent in real terms.

These and supporting figures appear to have prompted the only note of near-acerbity in the committee's report — that while the British Prime Minister has repeatedly stated that the science budget has been protected, "the health of university scientific research does not depend only on the science vote". ●

report* urges that waste be kept at reactor sites until a permanent repository is built to "avoid diverting the attention and efforts of the waste management agency away from the repository program toward provision of an independent interim storage facility".

Another sore point for environmental groups in the Senate bill is an amendment that Senator James McClure succeeded in attaching, which declares that the legislation itself represents reasonable assurance that a safe disposal method exists. Yeager says "It's a clear attempt to end-run several court cases and state laws". California, Oregon and several other states have passed laws restricting new power plants until such reasonable assurance exists.

To the industry, however, the amendment is nothing more than recognition of what they see as the obvious: the waste disposal problem is political, not technical. And here, the Office of Technology Assessment report backs the industry view. It found "no insurmountable technical obstacles" to development of geological repositories; rather, the chief obstacle is eroded public confidence, aggravated by a vacillating federal policy. President Carter, for example, reversed the previous policy of handling defence and commercial wastes separately; President Reagan reversed the Carter policy. Presidents Reagan, Carter and Ford each changed the number of sites under study for geological repositories and their construction schedules.

If a bill does emerge from Congress this year, it will almost certainly contain provisions to end this instability. Both Senate and House versions provide for long-term funding through a surcharge on nuclear electricity, which could raise \$300 million in the first year.

Both Senate and House versions also set up mechanisms for state participation in the site-selection process. The federal government's insensitivity to the concern of the states in previous siting decisions is viewed as a major factor in the loss of public confidence in the programme.

The chances that Congress will act this year are, according to all parties involved, better than they have been in past years. In 1980, both the Senate and the House passed bills, but failed to work out a compromise. This time, they start closer.

According to a staff member of Morris Udall on the House Interior Committee (Udall introduced the House bill), "there's no reason we can't get a bill except time". The prediction is that if the bill reaches the full House by mid-July, it should pass.

But passage may ultimately hinge on the complex politics of the House committees. No fewer than three have asserted jurisdiction over the measure, which is now tied up in the Energy and Commerce Committee. **Stephen Budiansky**

*Managing Commercial High-Level Radioactive Waste (Office of Technology Assessment, May 1982).

International disarmament

Palme's palm

A nuclear-weapons-free zone in Europe and greater United Nations (UN) power to prevent hostilities in developing countries are the chief recommendations of the Independent Commission on Disarmament and Security Issues whose report, *Common Security: a programme for survival*, was published last week. Although neither recommendation is new, Dr David Owen MP, former British Foreign Secretary, and Sir Shridath Ramphal, Secretary-General of the Commonwealth Secretariat, who presented the report in London, argued that their revival now is timely.

So, partly by design and partly by accident, was the publication of the report. The original intention was to produce recommendations for the UN Special Session on Disarmament which began this week. But the commission now also hopes that its document will inform the Strategic Arms Reduction Talks (START) due to begin in Geneva on 27 June. Dr Owen is optimistic that the South Atlantic conflict will focus attention on the recommendation for a UN fact-finding mission which could be called in by a country fearing imminent aggression by a neighbour.

The report comes after more than a year's deliberation by 17 distinguished politicians from as many countries under the chairmanship of Olof Palme, former prime minister of Sweden. The task of the commissioners, each of whom was chosen for their understanding of international affairs and not as a national representative, was to prepare a report on the consequences of the arms race along the lines of the Brandt report on economics.

The result is an analysis of global conflict and tension which embraces the effects of nuclear arms build up on North-South as well as East-West relations and the effects of the increasing sophistication and strength of conventional forces on relations between developing countries. The commission believes that the risk of a devastating war is increasing in spite of attempts at arms limitation. It says that national security can no longer be assured by military means.

The idea of a limited nuclear war and the conventional strategies of the NATO and Warsaw alliances on the early use of strategic nuclear weapons are challenged. The commission's answer is to create a nuclear-weapons-free zone in Europe by negotiating parity in conventional forces at reduced levels.

Over the past eight years, the report says, developing nations have been drawn into the arms race, while vertical proliferation in the five recognized nuclear powers has increased the probability of horizontal proliferation. Industrial nations have also made matters worse by selling sophisticated conventional arms to developing countries, making the escalation of local

disputes more likely. The commission is particularly critical of the high level of military spending by nations that can least afford it. Security for developing nations, particularly those with small populations whose borders are in dispute, must be assured by other means — hence the recommendation to revitalize the original intention of the UN charter by increasing the powers of the Security Council to preempt conflicts.

The commission's "programme of action" is divided into short and medium-term measures, for implementation within two and five years respectively. The programme includes measures to reduce nuclear and conventional forces in the NATO and Warsaw alliances, a nuclear-weapons-free zone, a comprehensive chemical weapons disarmament treaty, agreement on guidelines for conventional arms transfer, a substantial reduction in military spending and the transfer of military scientists to civilian research.

Little advice is offered on how to achieve these measures. But the commission is clearly hoping that the chief impact of its report will be to focus increasing public concern. **Judy Redfearn**

Italian science policy

New consensus

Venice

Italian science policy appears at last to be getting into gear, despite the almost annual changes of government that confound most long-term planning in this country. The reason? Politicians here have reached a consensus close to the French position that research is necessary to pull Italy out of its economic crisis.

This consensus is beginning to survive the rise and fall of governments, so the present science minister, lawyer Giancarlo Tesini, who has been in post for a year now in President Giovanni Spadolini's shaky five-party coalition, has been able to take some action.

One of his key moves has been to achieve five-party agreement on a reform of the Italian national research council (CNR) which until recently has dominated Italian government research and development both inside and outside universities. "CNR is a major problem of the Italian science ministry", Tesini said here last week, where he is attending a scientific meeting.

Broadly, Tesini wants to shift CNR, with most of its laboratories in universities, into a closer relationship with industry. The reform would put industrial scientists into key CNR positions and streamline an organization which is widely regarded as being massively bureaucratic — so bureaucratic, in fact, that in 1980 it failed to spend an important part of its budget concerning the Italian space programme. For its pains, the result in 1981 was a budget cut of 10 per cent in current lira (much more in real terms) to the present figure of

around 420,000 million lira (£180 million).

Under the previous government, CNR also lost control of university research (that is, research in universities outside CNR laboratories), which is now in the hands of the national council of universities (CUN), where the same professional groups take similar decisions but outside the CNR bureaucratic structure.

Tesini's reforms, now before parliament, would revitalize the crumbling structure of CNR. Already CNR is spending an increasing part of its budget on applied projects, the latest list of seven amounting to 300,000 million lira over five years. (Two examples of these projects are an effort to increase Italian agricultural productivity, particularly in the south, since Italy is a net food importer; and a recently completed project to develop an electronically-controlled fuel-efficient engine in collaboration with Alfa Romeo.)

But Tesini does not want to make CNR entirely into an organ of industrial research. "It would be dangerous to create a gulf between university work and CNR" he says.

Now the future of CNR is in the hands of parliament, but in the nature of Italian politics it may be two weeks or a year before the vote is taken on the Tesini reforms, by which time his government may well be out of office and a new minister in place to take the credit.

Robert Walgate

Humid tropics

Cautious growth

Washington

In a report released last Thursday, the National Academy of Sciences has recommended cautious but expanded development in the humid tropics. The study, commissioned by the US Agency for International Development*, found that concern over the environmental consequences of tropical deforestation is legitimate, but often overstated, and moreover that development is inevitable. Ninety per cent of the world's population increase from now to the end of the century will take place in the tropical countries — an addition of 1,500 million people.

One of the main messages of the report is that despite the inherent difficulties in managing tropical areas — and the study recites them all, including annual rainfall of 5–35 feet, tremendous species diversity, and poorly understood ecology — new technologies are appearing that can get around these problems.

According to Dr Pedro Sanchez of North Carolina State University, a member of the panel that prepared the report, some myths about tropical soils need to be dispelled. He points out that "there's nothing fundamentally different in the agronomy between the humid tropics and the south-eastern United States. Contrary to myth", he adds "tropical soils though often infertile and acid are not fragile. They are

Private help

Washington

In a move that will help fill the void left by the Reagan Administration's cutbacks in environmental research, a private group — the MacArthur Foundation — has stepped in to offer \$15 million for a new research organization. The Institute for World Environment and Resources will concentrate on issues in global ecology such as species loss, inadvertent climate modification, desertification, deforestation and population.

A key government agency that had been following long-term global issues, the Council on Environment Quality (CEQ) was cut back to a bare-bones staff as soon as President Reagan took office. The director of the new institute, James G. ("Gus") Speth, was chairman of CEQ under President Carter.

The institute, which expects to begin operations by October, will however attempt a broader and more scientific approach than CEQ has taken. It seeks to bring together scientists from the physical, biological and social sciences to work on interdisciplinary problems of significance to public policy.

Although the institute will be based in Washington, roughly one-quarter of its \$4 million annual budget is expected to go to established research contracts with university centres of expertise in the environment and resource fields.

Stephen Budiansky

not especially low in organic matter nor especially prone to erosion. A lot of the erosion pictures in the popular press are 'civil engineering erosion' pictures taken around roads and drainage systems".

Sustained agriculture on cleared land does, however, require careful management and new technology, in particular continuing application of fertilizer. Sustainable agriculture, as opposed to traditional shifting cultivation, is considered the key to attaining the greatest benefit with the least deforestation and thus the least environmental disruption.

Still, much may be possible through the fine-tuning of indigenous technologies. For example, the traditional slash-and-burn method of clearing turns out to be much easier on topsoil than are bulldozers, and furthermore provides free fertilizer in the form of nutrient-rich ash.

Dr H. Garrison Wilkes of the University of Massachusetts, Boston, another panel member, points out that too little has been done to exploit and improve on tropical germ plasm resources. "The amount we know about cowpeas," he says, "or other tropical crops such as casaba, palms, tropical cabbages is very small — so the potential for genetic improvement is tremendous."

Stephen Budiansky

*Ecological Aspects of Development in the Humid Tropics (National Academy Press, Washington DC, 1982).

Soviet agriculture

Food for all?

The Soviet Union's new food production programme designed to make Soviet food production match demand was announced last month at a plenary meeting of the Central Committee and introduced in a major speech by first Secretary Leonid Brezhnev himself.

To avoid emergency purchases from the non-Socialist world while this drift from agricultural to industrial employment continues will demand a major investment in agricultural research. Several lines of investigation are specifically mentioned in the programme, but their implementation is formally entrusted to the leading Soviet scientific bodies — the Academy of Sciences, the Lenin Academy of Agricultural Sciences and the State Committee for Science and Technology.

According to the programme, however, the practical work is to be the responsibility, primarily, of the network of "scientific production associations" set up in the past few years to link research and development organizations with production enterprises. These associations are to form the basis for the production of high quality and hybrid seeds and plants of "superior reproductive quality" as well as the rearing of pedigree cattle for state and collective farms.

The targets laid down by the new programme are precise: varieties of winter wheat must be developed with a yield of not less than 80–90 q ha⁻¹, spring wheat of 45–60 q ha⁻¹, maize hybrids of 12–130 q ha⁻¹ on irrigated land and 80–90 q ha⁻¹ on non-irrigated land and peas of 40–45 q ha⁻¹ (1q = 100 kg). Fodder crops must yield 10,000–15,000 fodder units per hectare under irrigation and 5,000–6,000 fodder units without, while technology or fodder storage must ensure retention of at least 90 per cent of nutrients.

In addition to these precise targets, there are less specific directives. Research is to be directed at saving energy in soil conservation, the rational usage of water resources, anti-pollution measures and the mass examination and treatment of animals. Special attention is to be paid to the development and mass production of biological and chemotherapeutic veterinary pharmaceuticals.

To support this applied research, the two all-union academies, together with the republic academies of sciences, are to develop theoretical work in genetic engineering. This will include the breeding of new strains of plants, microorganisms and animals, the biotechnology of protein synthesis and biologically active substances. The academies will also be responsible for new pesticides and herbicides, growth regulators and similar sophisticated agricultural preparations, and for designing the industrial technology for their commercial preparation.

Vera Rich

US National Institutes of Health

New clinical trials

Washington

Dr James B. Wyngaarden has finally been sworn in as director of the National Institutes of Health (NIH), the US health research agency, and so has come out of the shadows where he has been hiding, in a sense, since his name surfaced as the Administration's candidate for the post last autumn. He has now begun interviewing candidates for those institutes that have no permanent directors at the moment (some of the posts have been vacant for months).

Last month Wyngaarden also held his first press conference in his new capacity, and the debut produced one serious slip — a statement that his personal belief favoured freedom of individual choice regarding abortion — which was immediately attacked by anti-abortionists, who are very active in Washington these days. He also said, speaking personally, that he would have “no problem” with carrying forward NIH's research in animal *in vitro* fertilization to the human level. Whether NIH should sponsor research on human *in vitro* fertilization has been a controversial subject — so controversial that possible government guidelines have been pending, but not issued, since the time of the Carter Administration.

But when he spoke officially, Wyngaarden indicated his views for the future of NIH — which he has had ample time to work out in the long months of warm-up for the job. He said he wants to keep the present balance of activities, particularly the level of 50 per cent of NIH funding allocated for basic science including investigator-initiated grants. He also favours continuing use of training grants and fellowships as a means of producing good basic biomedical scientists. He said he agreed “fundamentally” with the stabilization programme meant to insulate NIH from budget swings that was introduced by his predecessor, Donald K. Fredrickson. Wyngaarden also defended peer review as a “brilliant creation” of one of his predecessors, James A. Shannon, and said that NIH were not at the moment well placed to embrace more institutes. All this was hardly surprising, given Wyngaarden's background in academic medicine at Duke University School of Medicine.

Wyngaarden did, however, express interest in starting new clinical trials. NIH still run several, but some have come under criticism for being very expensive and sometimes not useful. No new clinical trials have been initiated by NIH since 1978. But Wyngaarden said he was considering suggestions for new clinical trials in the cancer, cardiovascular and arthritis fields that might well be worth starting even though they would commit funds for many years and take money away from

investigator-initiated grants. New clinical trials would, of course, mean a change of the balance of activities of NIH. But such a change, if it comes, is some time off, as Wyngaarden has much to do now to get NIH running again. **Deborah Shapley**

Biotechnology

Yugoslav plan

Zagreb

Yugoslavia is planning a major drive in biotechnology, based on a projected new institute on the Dalmatian coast. The new scheme, which now awaits federal approval, has the backing of the government of the Croatian Republic, and is intended both to enhance the international prestige of Yugoslav science and to help the drive to develop science-based industry.

The present stagnation of the Yugoslav economy has made it impossible for science and industry to continue its recent course of dependence on foreign licences and the import of obsolescent technologies. Indeed last year's Congress of Self-Management stressed unequivocally the need for home-grown technology.

Some research institutes embarked on such a course several years ago. Thus the Jozef Stefan physics institute in Ljubljana supplies knowhow to a range of industries from medical technology to nuclear power and the Immunology Institute of the University of Zagreb has become a major earner of hard currency by means of sales of sera and sophisticated pharmaceuticals, including interferon.

The new emphasis on science-based industry, however, accords well with the desire of Yugoslav — and particularly Croatian — biologists to see their country take a lead position in biotechnology. During the academic year 1979–80 a group headed by Dr Marija Alacevic of the Department of Biotechnology of the University of Zagreb raised the possibility of founding an international institute for molecular genetics somewhere on the Croatian coast.

Several coastal towns were asked to tender for providing a site. Dubrovnik, which already boasts an Inter-University Centre of Postgraduate Studies, was tipped as favourite. Just before the closing date, however, Split made an intriguing counter-proposal. The institute, Split suggested, could be housed in the Villa Dalmatia, one of the residences of the late President Tito. The proposal now only awaits official ratification. Apart from symbolic implications, Split has other advantages including a long-standing academic tradition which, in 1974, culminated in the foundation of a university.

The choice of biotechnology seems to be dictated partly by economic considerations — the costs involved in biotechnology, while not negligible, are modest. Moreover, Yugoslavia has a long tradition in organic chemistry and can boast two

Nobel prize winners — Ruzicka and Prelog — in this field.

Supporters of the institute already speak of the projected institute as “international”. There is hope that the European Molecular Biology Organization (EMBO) will help but that would mean that Yugoslavia would first have to join EMBO — a proposal already formally made by the Yugoslav scientific community to the federal government. Yugoslav participation in international scientific research has not always been auspicious — although a founding member of CERN, for example, Yugoslavia had to drop out because it could not meet the financial contributions.

According to Professor Ivo Slaus, the chairman of the steering committee for the institute, even if no international support is forthcoming, the Croatian scientific community would still wish the institute to be “international” in the wider sense of the word. The Republic of Croatia has already allotted 15 million dinars (£188,000) for preliminary work and part of this sum will be spent on a conference next year to launch the new institute and to ensure that it does not duplicate the work of existing institutes (such as Szeged in Hungary).

Vera Rich

UK–Argentine cooperation

Shadows of war

The storm clouds over the South Atlantic are casting long shadows over scientific relations between Argentina and the United Kingdom. Scientific collaboration between the two countries had been growing but it now looks as though several promising projects may be blighted. The rumblings have already caused the postponement of the Sixteenth International Symposium on Remote Sensing of Environment, which was to have been held in Buenos Aires this week. Organized jointly by the Environmental Research Institute of Michigan and the Comisión Nacional de Investigaciones Espaciales, the conference was to have heard 200 papers. When the Falklands crisis erupted, many intended participants withdrew their papers and gave notice that they would not attend the symposium.

Scientific collaboration between Britain and Argentina — in the form of joint university projects, exchange visitors and fellowships — has been nurtured over the past few years by the Royal Society and the British Council. The Royal Society and Conicet (its Argentine equivalent) have had a reciprocal agreement for some ten years — 110 scientists a year have been exchanged. All this has now been put on ice. The Royal Society recently sent out a letter to scientists it had planned to sponsor on visits to Argentina saying that, in view of present circumstances, all exchanges should be halted.

The British Council has increasingly encouraged British academics to put out

feelers and develop reciprocal research projects with Argentina. Through the council's office in Buenos Aires, Argentine universities would suggest projects for possible cooperation and the council would then approach the universities in Britain. Travel expenses were paid by the visiting country's organization, the council or Conicet, and subsistence by the host. The council has now closed its office in Buenos Aires and recalled its staff.

When the Falklands crisis broke, eighteen jointly-funded university projects were under way. In the past year the British Council has sponsored 7 long-term scholarships, 36 visitors and 17 lecture trips. The numbers may not be great but the Royal Society, the British Council and many academics have expressed their profound regret that the present situation may put a big question mark over such agreements.

Several promising projects have been nipped in the bud. Imperial College and the Centro de Investigaciones Ópticas (CIOP), La Plata, have for the past eighteen months been discussing the setting up of a joint laser physics research project. Professor G.H.C. New of Imperial College recently paid a visit to Argentina to give a series of lectures and arrange future collaboration. As well as exchanging postdoctoral fellows on extended visits — beneficial for each party in providing highly specialist researchers free — it was also hoped that a British specialist would go out to Argentina to teach and act as a consultant at CIOP.

Professor J. Barber (Pure and Applied Biology, Imperial College) has also recently been to Argentina to coordinate a joint photosynthesis research project. The oceanography department at the University of Southampton has been making moves to coordinate research with Argentina, particularly in the area of oil exploration. Professor A.P.M. Lockwood has been closely involved in talks to set up training exercises particularly in his capacity as a delegate of TEMA (Training and Education for Mutual Advantage), part of UNESCO's International Oceanographic Commission.

Both Argentine and British scientists are concerned that the Falklands crisis will seriously damage opportunities for scientific cooperation. In particular, many Argentine scientists in Britain are worried that if they leave the country to attend conferences abroad, they may have problems getting back into Britain. In April, both Britain and Argentina introduced a visa requirement. A spokesman for the Home Office did say, however, that he could not foresee any problem if Argentine nationals applied for re-entry visas before leaving the country. What is clear is that many academics feel uncomfortable in a situation where politics is impinging on their research. As one who has recently visited Argentina said, "They're a wonderful country and wonderful people. It's very, very sad".

Jane Wynn

Australian telesatellites

Outback opened

Canberra

Australia will have its own domestic communications satellite by the end of 1985. In a statement to the House of Representatives on 6 May, the then Minister for Communications, Mr Ian Sinclair, announced that Hughes Communications International will build the space segment of a domestic satellite. The system, known as Aussat, will be owned and operated from the start as a commercial venture by Aussat Pty Ltd. Although Aussat is at present wholly owned by the federal government, the government intends to sell 49 per cent of its equity to the private sector as soon as practicable, in line with its free-market policy principles.

The contract with Hughes, the sealing of which seems imminent, specifies three satellites for the system — two in orbit and one on ground standby, with the option of a fourth satellite should demand justify it. The satellites will have an operational lifetime of seven years and function in the super-high frequency (gigahertz) range with four high-power and eleven low-power transponders. The net result will be a versatile satellite of limited capacity, primarily suitable for television networks. Provisional bookings with the European Ariane launch vehicle and with NASA's Space Shuttle or Delta rocket have been secured. The fixed-price package offered by Hughes, including the launching and construction of two ground control stations, will cost A\$166 million (£95 million). Of this, only A\$5.1 million will be spent on local technology, which is a sad reflection on the dearth of innovation in the Australian electronics industry.

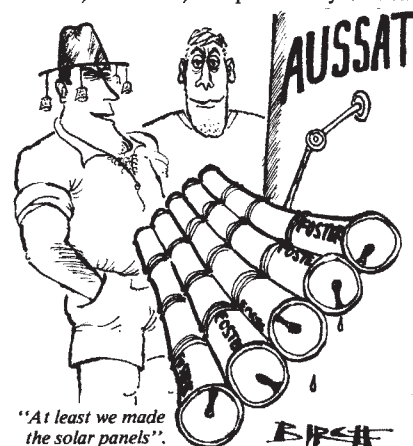
Aussat promises a glittering array of services including direct television broadcasting to widely scattered homes throughout the continent, business communications and remote educational and medical services. Both the School of the Air, plagued by poor reception, and the Royal Flying Doctor Service will get a new lease of life. The satellite will also bring telephone services to the outback as well as a back-up service for trunk lines between major cities. Unfortunately, the more imaginative aspects of satellite telecommunications such as "teleconferencing", direct radio contacts and electronic news gathering are ruled out in this satellite because of its frequency allocation and limited number of transponders.

The principal government user of Aussat will be the Australian Broadcasting Commission, which has reserved all the four high-power transponders on the first satellite for its Homestead and Community Broadcast Satellite Service. The beneficiaries will be farmers and station-owners who can afford A\$1,000 for the purchase of a 1.2-metre diameter recep-

tion dish. For the rest of Australian viewers, the satellite will bring more of the same; the Australian Broadcasting Commission is likely to increase the proportion of its programmes relayed in real time, rather than on film or tape, to its network. On the other hand, Aussat may facilitate the production of special-interest programmes for small groups such as ethnic communities, for which there is insufficient local appeal but which may be viable on a national basis.

Other public users will be Telecom, who will provide the remote telephony and business services, the Department of Transport, which will use the facility for aeronautical and marine communications and the state police departments.

The commercial television stations are expected to be the principal private users. There is, however, the possibility of using



the high-power transponders on the second satellite on a pre-emptive basis, in which case the fourth satellite will certainly be needed. Significantly, Aussat will provide the first opportunity for a private operator to compete with Telecom. At present, legislation ensures that Telecom has a monopoly in all landlines and terrestrial microwave links. However, Telecom has a statutory obligation to weight its charges for profitable services in order to cross-subsidize telephone services in rural areas. A competitor, not hampered by these obligations nor by having to contend with the growing union activism within Telecom, could under-cut Telecom for its business services via satellite and still make massive profits.

The substantial private equity in Aussat raises political questions of regulating ownership, access and frequency allocation. The satellite, by enhancing television networking, will undermine the position of commercial stations not having access to it. Consequently there is a real danger that the media, now already in a few private hands, will be even further concentrated. Another problem to be tackled is the possible piracy of the downlink beam by neighbouring countries or illegitimate access to international and overseas databanks. Australian legislators will have to resolve these problems with little by way of precedent.

Vimala Sarma

CORRESPONDENCE

A history of the Falkland Islands

SIR — In your leading article referring to the Falkland Islands conflict (see *Nature* 8 April, p.480), it is stated that the only claim of Argentina to the ownership of the islands is the proximity to the Argentine coast. Since we consider this statement to be in error we give here a detailed account of the historical events and the cause of the present conflict.

The discovery: The strongest evidence available indicates that the discovery of the islands can be attributed to the crew of the ship "San Anton", a member of the Spanish expedition led by Hernando de Magallanes (Magellan). With the name of "Islas de Sanson" (an abbreviation and slight modification of the ship's name) the islands are depicted in several Spanish maps from 1522 to 1590, as well as in the Italian map of Agnese, of 1536, where the Magellan route is indicated.

Naturally, Spanish dominion over the islands was proclaimed (in accordance with Papal bulls and treaties with Portugal) and in 1580 a garrison was settled near the Magellan Strait having jurisdiction over the mainland and the nearby islands.

In 1619, on a map made under the direction of the Dutch sailor Sebald de Weert, the islands appear with the name of "Sebalдин islands". The first British descriptions were published in 1622 but even British scholars are doubtful about these reports.

However, the British sea rover William Dampier was there by 1648 and in 1690 John Strong gave the name "Falkland Sound" to the strait between the two main islands, a name that in the English literature was later extended to the whole archipelago.

The occupation: The islands were first occupied in 1764 by the Frenchman Louis Antoine de Bougainville who established a small colony in a place he named Port Louis. Since most settlers were from the French port of St Malo the islands took the name of "Iles des Malouines", which in the Spanish literature became "Islas Malvinas".

Once Spain realized that the French had settled there the Crown made the appropriate claims to France and in 1767 the French left. A small Spanish garrison subsequently remained in Port Louis, which took the name of "Puerto Soledad".

In the meantime (1765) a small group of people from Britain had settled in a place which they called Port Egmont. Once again, Spain made the respective claims and since the British did not want to leave peacefully they were expelled by force in 1770. The diplomatic conflict that started ended by an agreement between Britain and Spain in which the British were reinstalled in Port Egmont in 1771 and left "spontaneously" in 1774. The British occupation lasted four years in all.

In 1811, after 44 years of regular occupation, the Spanish garrison was sent to Montevideo because of the War of Independence that had started in Buenos Aires in 1810. This war led to the Declaration of Independence in 1816, following the North American example.

Argentina, as heir to the Spanish possessions, took charge of the Islas Malvinas

in 1820 and left a representative of the Government of Buenos Aires.

In 1825 Britain recognized the independence of Argentina and signed a treaty of peace and commerce without questioning the Argentine possession over the islands.

In 1829 the administration of the island was reorganized and a governor was sent from Buenos Aires. He settled there with his family.

On 2 January 1833 John James Onslow, commanding the British frigate "Clio", dismissed the Argentine authorities by force. The British forces stayed there until 2 April 1982.

The claims: The Argentine government considered the British action to be plunder and immediately made the corresponding claim. Since then this claim has been repeated by the Argentine government (regardless of its political affiliation) at every convenient opportunity but no satisfactory answer has been obtained.

The creation of the United Nations opened a new possibility, particularly when in 1960 it decided to end colonialism. Britain spontaneously presented the "Falkland Islands" as a "non-autonomous territory", an expression that replaced the old term "colony" (Resolution No. 1514, XV General Assembly).

Since Argentina claimed the property of the islands, in 1965, after patient diplomatic procedures, the 20th General Assembly issued Resolution No. 2065 (approved by 94 votes in favour, none against and 14 abstentions, Britain among them) in which both Britain and Argentina were invited to solve the conflict in a peaceful way.

Conversations started and during this time Argentina has steadily contributed to the improvement of the living conditions of the islanders which were neglected by the British government. Schools were improved, an airport was built (the only one in the islands) and twice-weekly air communication with the continent through an Argentine airline was started, thus ending the island's long isolation. Free health assistance and education on the continent were also offered to the islanders. It is important to stress that the islanders were primarily dependent on the "Falkland Islands Company" which controlled every aspect of their lives: production, commerce and even food supply. The company also owns most of the useful land.

At first Britain seemed to accept a diplomatic solution and even stated that she would transfer the islands to Argentina provided the interest of the 1,800 inhabitants was taken into consideration. However, Britain subsequently paid no further attention to the subject and no more steps were made in the negotiation.

The conflict: The present conflict originated as follows: At Grytviken, San Pedro Island, a Malvinas Islands dependence, there was on old and abandoned whaling factory. An Argentine scrap dealer made the proper arrangements with the owner of the factory to dismantle it and sell it for scrap. The arrangements were made according to British law and with the British authorities' approval. Nevertheless,

once the Argentine workers were at Grytviken, they were considered to be "invaders" and a warship was sent to clear them out. Argentina in turn first sent another warship to protect the workers and then occupied the islands. The recovery of the whole archipelago was made without any harm to the British. Not a single drop of British blood was shed but four Argentine people lost their lives.

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Logarithmic SI

SIR — I have for some time made private use of a notation for expressing numbers and measurements that others might perhaps also find useful. It was suggested to me by the symbol "pH".

The first step is to let the prefix p mean "antilog to the base 10". So p0 is 1, p6 is 1,000,000; 2,000 is 2p3 or, to one decimal place, p3.3. For, say, 0.02 there is a choice of p2.3, p1.7 or 2p2. (More generally, p may be regarded as p¹, giving p^{0.2} as 2, p^{1.2} as 100, p^{2.2} as p100, and so on — so p^{9.9} is a very large number.)

The next step is to let pQ0 mean the SI unit value of some quantity Q; this generates a notation that is particularly useful for very large or very small measurements.

My own practice is to use pL0, pM0 and pT0 for 1m, 1kg and 1s respectively. Area, volume, speed and acceleration may conveniently be written pL², pL³, pL* and pL** (I say 'pL stars' — c.f. x); the two latter pL/T and pL/T² alternatively. For other units I use the SI symbol itself — 1 joule is pJ0. (I also write pD0 for 1 kg m⁻².)

As examples, electron mass and radius (m_e and r_e) are pM30.0 and pL14.6; the solar mass pM30.3 and the Solar System's diameter about pL13; the speed of light pL*8.5 and of continental drift about pL*9; and a year is within 1 part in 500 of pT7½.

This notation is particularly practical in that it avoids the awkwardness of the half-sized superscript (2×10^{30} kg), while getting round the somewhat clumsy SI prefix system, which in general usage has caught on only rather patchily.

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Support the Zoo

SIR — The article by Jane Wynn in *Nature* 13 May (p.97) points up what seems to me to be an obvious anomaly. Kew Gardens is (rightly) given enough Exchequer support to enable it to charge an entry fee of 10p, and to have over 1 million visitors a year. London Zoo, which has similar functions, is forced to try to charge an "economic" entrance fee and hence price itself out of existence. Why the difference?

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NEWS AND VIEWS

The oncogenic circle closes

from Peter W.J. Rigby

A LITTLE over a year ago I discussed in these columns¹ recent work from the laboratories of Geoffrey Cooper (Harvard Medical School) and Robert Weinberg (MIT) which showed that the NIH3T3 line of mouse fibroblasts can be morphologically transformed by transfection with DNA from chemically induced and spontaneous tumour cell lines. The use of DNA-mediated gene transfer techniques to define and characterize the oncogenes of non-virally induced tumours marked a significant step forwards in molecular oncology. Yet in March 1981 many questions remained. Nothing was known of the nature of these oncogenes or of their products and there was much speculation as to the relationship between these cellular transforming genes and proto-oncogenes, the cellular homologues of the oncogenes carried by strongly transforming retroviruses². Now an extraordinary series of reports from the groups of Cooper and Weinberg and of Michael Wigler (Cold Spring Harbor Laboratory), Mariano Barbacid, Edward Scolnick and Douglas Lowy (National Institutes of Health) has precisely identified several cellular oncogenes and their products and has shown that in two cases, the genes detected by transfection are proto-oncogenes.

Cellular oncogenes were first characterized in terms of their sensitivity to restriction enzymes. It was shown that the DNA of different tumour types transferred different oncogenes but that within a tumour type, for example, mammary carcinomas, the same oncogene was transferred by the DNAs of independent tumours even when the tumours arose in different species, suggesting that the oncogenes are specific for a particular cell type. This idea has now been extended by Cooper's group in a study of twenty B- and T-lymphocyte neoplasms of human and murine origins³ that shows that different oncogenes are activated in tumours derived from cells of the same lineage at different stages of differentiation. Either different oncogenes are highly susceptible to activation at different stages of differentiation or the

expression of a particular oncogene is required to transform cells of a given differentiation state. This latter explanation, of target specificity within a lineage, has parallels in the interactions of defective avian leukaemia viruses with their target haematopoietic cells⁴.

The expected application of recombinant DNA techniques has now occurred, the favourite target being the human bladder carcinoma oncogene which has been cloned by the groups of Barbacid, Weinberg and Wigler⁵⁻⁷. The gene is contained within an approximately 6.6 kilobase (kb) *Bam*I fragment and Barbacid's group has further localized the transforming activity to a 3.0 kb *Sac*I fragment⁸. Southern blotting experiments show that NIH cells transformed by bladder carcinoma DNA always carry this fragment of human DNA and that the allele of the gene carried in bladder carcinoma cells is not detectably rearranged in comparison with the allele present in normal human DNA. Barbacid's group has cloned the homologous sequence from normal human fetal liver DNA and shown that within the biologically active 3.0 kb *Sac*I fragment there are no differences detectable by restriction enzyme or heteroduplex mapping⁸. The fact that the T24, J82 and EJ lines of bladder carcinoma cells transfer the same oncogene is not surprising because at a recent meeting in Squaw Valley, California*, Wigler reported restriction site polymorphism and isoenzyme data which show that the T24 and J82 bladder carcinoma lines are identical and that the EJ line is, at the least, very closely related. At the same meeting, Cooper reported the cloning of another oncogene transferred by DNA from bursal lymphomas of the chicken. The striking aspect of this gene is its small size; only 1.8 kb of DNA are required for biological activity and much of this segment is comprised of highly repeated sequences. The region that hybridizes to mRNA is very short, approximately 0.3 kb, suggesting that the gene product will be a rather small polypeptide.

The relationship between cellular oncogenes and proto-oncogenes has been studied by all the groups. About fifteen proto-oncogenes are now known and they have acquired a genetic notation of their own⁹. Each gene has a three letter symbol

derived from the name of the virus in which it was first defined. Thus *abl* is the transforming gene of the Abelson murine leukaemia virus, *fes* the gene of feline sarcoma virus, *myc* the gene of avian myelocytomatosis virus (MC29) and so on. The prefix *v-* indicates the intronless version of the gene carried by the retrovirus, *c-* the homologue in normal cellular DNA.

Cooper and Weinberg and their colleagues have used cloned retrovirus *onc* gene probes in Southern blotting experiments to analyse the DNAs of NIH cells transformed by a variety of cellular oncogenes^{10,11}. The mouse homologues of the *onc* genes are always detected but if the donor DNA is of a different species, say human, and if the transferred cellular oncogene is related to the retrovirus probe, the question remains whether additional fragments corresponding to the transferred human gene will be detected. To the great delight, and probably even greater surprise, of all retrovirologists, such relationships have been revealed in the first series of experiments. Weinberg's group has shown that the EJ bladder carcinoma oncogene is homologous to *c-Ha-ras1*, the cellular equivalent of the transforming gene of Harvey murine sarcoma virus (HaMSV). Cooper's group report similar data for the EJ and J82 oncogenes but even more surprisingly show that the oncogene transferred by the DNA of the LX-1 human lung carcinoma line is homologous to *v-Ki-ras*, the transforming gene of the Kirsten murine sarcoma virus (KiMSV). Wigler (Squaw Valley meeting) has also reported the same results. Weinberg's data show that a novel fragment hybridizing to *c-Ha-ras1* is present in all NIH cells transfected with EJ or T24 DNA and in secondary transfectants. Moreover, if DNA from a secondary EJ transfectant is cleaved with *Bam*I, an enzyme known not to inactivate this oncogene, and transfected into NIH cells, one again sees a novel fragment homologous to *c-Ha-ras1*. These novel fragments are also detected by the cloned oncogene and both probes hybridize to the same fragment of normal human DNA. The groups of Barbacid and Weinberg have compared the cloned *ras*

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*The Cetus-UCLA Symposium on 'Tumor Viruses and Differentiation', was held at Squaw Valley, California, 21-27 March 1982.

gene and the cloned bladder oncogene^{8,11}, and have shown that the sequence homology resides within the 3.0 kb *SacI* fragment required for biological activity. Quite unexpectedly, Barbacid's group also demonstrates weak homology between the T24 oncogene clone and *v-myc*, although this homology lies within a 0.85 kb *SacI* fragment not required for biological activity.

These data show clearly that the 3.0 kb *SacI* fragment contains the bladder oncogene and that there is homology to *ras*, but do not prove that the two genes are identical; they could merely be closely linked or interdigitated. However, analyses of expression make this latter possibility highly unlikely. Wigler's group showed⁷ that the T24 oncogene detects an approximately 1.2 kb cytoplasmic RNA in T24 cells and in NIH cells transformed by the T24 oncogene but not in untransformed NIH cells. Weinberg's group has now shown¹¹ that this RNA, and a 5.1 kb transcript, hybridize to both the cloned oncogene and *c-Ha-ras1*, and Barbacid's group reach the same conclusion⁸ using the cloned oncogene and *v-bas*, the transforming gene of the BALB murine sarcoma virus, which is identical to *ras*. Final evidence for identity comes from studies at the protein level. Scolnick's group has extensively analysed the protein encoded by *v-ras* and has raised a panel of monoclonal antibodies against this phosphoprotein of apparent molecular weight 21,000 (p21). Barbacid's and Cooper's groups show^{8,10} that NIH cells transformed by the bladder oncogene contain higher levels of p21 than do untransformed cells and Weinberg's group mention that they have similar data¹¹.

Thus the cloned human bladder carcinoma oncogene is homologous to *ras*, the transforming gene of HaMSV; cells transformed by the bladder oncogene express an RNA which hybridizes to both oncogene and *ras*; and such transformed cells contain elevated levels of the protein encoded by *ras*. The inescapable conclusion is that the bladder oncogene is an allele of the normal human homologue of *ras*, activated in a subtle, yet to be determined way. If this is the case, then the normal human gene, if artificially activated, should transform NIH cells. Lowy and Scolnick and their colleagues have shown that this does indeed occur. As part of their wide-ranging studies of the *ras* system they have cloned four segments of human DNA with homology to *ras*, one of which, human *c-Ha-ras1*, is very similar to the rat *c-Ha-ras1* probe used by Weinberg. This cloned segment does not transform NIH cells. If, however, the 3.0 kb *SacI* fragment from this clone, which is, of course, equivalent to the 3.0 kb *SacI* fragment of the bladder oncogene, is linked to the long terminal repeat (LTR) of HaMSV, the resultant chimaeric molecule transforms. Similar activation can be achieved using the LTR of feline sarcoma

virus. The transformed cells contain within their DNA the human *c-Ha-ras1* gene linked to the viral LTR and express the p21 protein.

A normal human gene can thus be activated either by some as yet undefined change occurring during the induction or progression of a human tumour or by linkage to the control sequences contained within a retroviral LTR. The consequence of this activation is the expression of elevated levels of p21 which is also the transforming agent of a murine retrovirus. It should be noted that transformation does not result simply from the expression of p21 but from elevated expression of this protein. Both the corresponding RNA and p21 are found at low levels in normal human cells (ref.12 and Wigler at the Squaw Valley meeting). The notion that cancer is a disease caused by dosage, that it results from elevated expression of normal gene products, is thus further supported.

Cooper and Weinberg have also taken a separate approach to the identification of the products of cellular oncogenes^{13,14}. NIH cells transformed by a cellular oncogene can be used to induce tumours in young mice and sera from the tumour-bearing animals can then be employed to immunoprecipitate labelled extracts from cells transformed by that oncogene. Any proteins specifically immunoprecipitated are likely to be either the product of the oncogene or a protein directly induced by its action. Sera taken from animals bearing tumours induced by NIH cells transformed by other oncogenes should not recognize this protein and, conversely, the protein(s) should not be present in extracts of cells transformed by other oncogenes. In this way, Weinberg's group has identified a non-glycosylated phosphoprotein of apparent molecular weight 185,000 synthesized in NIH cells transformed by the oncogene of nitrosoethylurea-induced rat neuroblastomas. This protein is also found in cells transformed by DNA from a rat glioblastoma but this tumour was obtained by the same protocol as the neuroblastomas and derives from a similar tissue type. Cooper's group has concentrated on cells transformed by the oncogene of the human mammary carcinoma line MCF-7. The antisera

obtained immunoprecipitate a non-phosphorylated glycoprotein of apparent molecular weight 86,000 (gp86) and often a protein of apparent molecular weight 19,000. Restriction enzyme digestion experiments have shown that the MCF-7 oncogene is closely related to the oncogene of mouse mammary carcinomas induced by mouse mammary tumour virus (MMTV) or by dimethylbenzanthracene (DMBA). In agreement with this result, gp86 is precipitated from extracts of cells transformed by the MCF-7 oncogene by sera from mice bearing tumours induced by cells transformed by DNA from MMTV- and DMBA-induced mammary carcinomas and by sera from mice bearing primary MMTV-induced carcinomas. gp86 cannot be detected, however, in cells transformed by DNA from MMTV- or DMBA-induced carcinomas, or in primary MMTV-induced carcinomas or in MCF-7 cells. Thus while the connection between gp86 and the cellular oncogene is not clear-cut, it seems likely that this protein will turn out to be either the product of the mammary carcinoma oncogene or to be directly induced by it.

Despite the astonishing advances that have been made in the past year, many important questions remain unanswered. First, what is the general validity of the NIH3T3 cell assay for cellular oncogenes? Almost all the work has used this cell line simply because it transfects extremely efficiently. These cells are not normal however, and could be described as teetering on the edge of transformation. Many are the tales of those who, in their first experiments with these cells, succeed in generating many foci without the application of any DNA! In more scientific terms, these cells transform with kinetics indicating that only a single event is required, whereas most data suggest that natural carcinogenesis is a multi-step phenomenon. However, Sager and her colleagues have recently shown that the bladder carcinoma oncogene will efficiently transform CHEF/18 cells¹⁵. This Chinese hamster embryo fibroblast line has been extensively studied by Sager's group and their data indicate that transformation in this system normally requires multiple events. They conclude that the activated bladder oncogene is the end product of the multi-step carcinogenic process. Weinberg's group has shown¹⁴ that the rat neuroblastoma oncogene will transform Rat-1 cells, a line of rat fibroblasts that (in my experience at least) is more normal than NIH3T3 cells. The development of additional cell systems for the assay of oncogenes will be important but in the final analysis we need to know whether an oncogene will transform its apparent target cell type. However, the development of efficiently transfectable cells derived from a wide variety of specialized tissues will be technically difficult.

The second major question is whether

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oncogenes detected by the NIH cell assay are the primary causative agent of the tumour from which they are derived or is their activation the end result of a complex series of genetic events? That this latter possibility may be correct is suggested by work on bursal lymphomas of chickens. If chickens are infected with lymphoid leukaemia viruses, avian retroviruses which do not carry an *onc* gene, bursal lymphomas arise with long latency periods. Analysis of the DNA of such tumours shows¹⁶⁻¹⁸ that the retrovirus integrates so that its LTR, the segment of the viral genome containing both a promoter and an enhancer, is adjacent to *c-myc* and transcription of this proto-oncogene is thus activated. However, if bursal lymphoma DNA is used to transform NIH3T3 cells the oncogene transferred is not *c-myc*¹⁹ but the

gene now cloned by Cooper and discussed above. By analogy with this situation the primary target during the induction of bladder carcinoma may not be the gene which has been cloned. If so, quite different experimental approaches will be needed to identify it, and the oncogenes of the many tumours not active in the NIH cell assay.

There can, however, be no doubt that the work discussed here is a very significant landmark in the development of our understanding of carcinogenesis. The application of modern genetic and immunological techniques has led to insights undreamed of even a few years ago. The argument that fundamental research in molecular biology is irrelevant to the cancer problem is surely truly dead and buried. □

In, out and about meteorites

from N.J. McNaughton and P.K. Swart

At a recent conference* in Houston much attention was given to the search for evidence of what must have been (if, indeed, it did occur) the most dramatic interaction between the Earth and an extraterrestrial object — the impact of the 10 km diameter meteorite that Alvarez has proposed was responsible for the mass extinction of the dinosaurs and many diverse faunal and floral groups at the end of the Cretaceous period. Alvarez based his hypothesis on the observation of anomalous iridium levels at the Cretaceous-Tertiary (K-T) boundary (*Science* 208, 1095; 1982) and, judging from the conference, a growing number of scientists are now committed to his view.

Theoretical and experimental studies clearly demonstrate both the catastrophic consequences of a meteorite impact in the ocean and the global dispersal of ejecta. A massive 5 km high tidal wave (Ahrens and O'Keefe, Caltech) would be produced and disrupt food chains by extensive stripping of sediments and silting. A worldwide dust layer would be raised by the impact. Atmospheric temperatures would fluctuate rapidly as a result of the production of volatilized steam and ejecta from the impact and then drop as ejected dust obscured the Sun. To understand how the possible extinction mechanisms operated, scientists have studied the isotopic composition of the ejecta, particularly the layers of sanidine, glass spherules and microtektites which coincide with the extinct marine phytoplankton in the deep-ocean sediments.

Epstein (Caltech) showed that the sanidine spherules have a terrestrial ratio of ¹⁷O to ¹⁸O, but their unusually heavy ^{δ¹⁸O}

may be due to either exchange with atmospheric oxygen at the very high temperatures that would accompany impact, or diagenesis. DePaulo's group (University of California, Los Angeles) also noted the high ^{δ¹⁸O} of the K-T boundary and further suggested from Sr and Nd isotopic studies that the layer resulted from an oceanic impact in which most of the material is of terrestrial origin, but only a small proportion is in fact derived from the local sea floor. Shaw and Wasserburg (Caltech) argue from their Sr and Nd isotopic data that the sanidine spherules are of non-oceanic derivation. Although this apparent conflict undoubtedly arises from the different types of samples studied, all the isotopic evidence supports the view of Ahrens and O'Keefe that the meteoritic component in the ejecta is minor.

The impact site has not been identified as neither a continental nor an oceanic crater of the appropriate size and age has been found. An oceanic impact would seem more likely, however, because a greater proportion of the Earth was ocean at that time. Over half the late Cretaceous oceanic crust has now disappeared into subduction zones, and the lack of evidence of an oceanic crater is thus not necessarily a problem for the hypothesis.

While the ejecta of meteorite impacts on Earth are sought for further study, the debris from similar collisions on other planets may already be represented in our meteorite collections. The achondritic shergottites, nakhlites and chassignites (SNC), represented by only nine of the several thousand meteorites held in museum collections, are argued to have an origin in meteorite impact with asteroid or planet. The SNC are distinguished by

relatively young ages, 1.3 billion years, unique but consistent oxygen isotope composition (Clayton and Mayeda, University of Chicago), unusual 'cumulated' texture indicative of crystal settling under gravity, and complex chemical composition. The most obvious initial source, the asteroids, are precluded as these have insufficient mass to sustain the igneous activity that could account for the young age. Sufficient melting could occur, however, as a result of collision between the meteorite and either an asteroid or a planet such as Mars.

Opponents of the idea that SNC meteorites are martian ejecta argue that the proposed 100 m diameter SNC parent body could not reach the required escape velocity of 5 km s⁻¹. An impact of sufficient size would vaporize, melt and crush to fine dust most of the ejecta (Singer and Melosh, University of New York) and, in any case, the resultant fragment should be highly shocked; but this is not observed. Singer and Melosh propose instead that SNC meteorites represent 'pockets' of melted rock caused by impacts on large asteroids. The proponents of the martian impact theory counter that such melt sheets cannot produce the required textures and point to chemical similarities between martian soil and SNC meteorites. Nyquist (Johnson Space Center) argues that the problem of reaching the escape velocity may be overcome if incoming projectiles were to strike the surface of Mars at a shallow angle, so that debris ricocheted away at high velocity. Such impacts would leave characteristic 'butterfly' pattern craters which are, indeed, plentiful on Mars. A more rigorous chemical and isotopic comparison with martian data obtained from the Viking programme or, in the future, with returned samples, will perhaps resolve the origin of the SNCs.

To understand chondrule formation and meteorite agglomeration, meteoriticists are increasingly turning to the stable isotopes of the light elements for new insight. Clayton and Mayeda (University of Chicago) demonstrated that two separate processes are recorded in the oxygen isotope composition of density separates from Murchison. The heavy-density separates, ostensibly from the chondrules, and olivine-pyroxene and spinel separates define a line of slope around 1 on a plot of ^{δ¹⁷O} versus ^{δ¹⁸O} which is best explained by high-temperature mixing and exchange between ¹⁶O-rich solids with ¹⁶O-poor gas. The low-density and calcite separates define a line of slope around 0.5 which diverges from the mixing line and is explained by low-temperature alteration of the pre-existing materials by water from the same gaseous reservoir that produced the mixing line. That the carbonate, in particular, is formed significantly later

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than the chondrules and the high-temperature silicates is further supported by new Rb/Sr data for Orgueil, which suggest an age gap as large as 160 Myr. (Maddougall and co-workers, University of California, San Diego). The secondary nature of the carbonates has important implications, not the least of which is its enrichment in carbon-13 compared with other co-existing carbon-bearing phases.

Several workers have attempted to separate the carbonaceous components by HF/HCl demineralization of the meteorites. Kerridge (University of California, Los Angeles) found that stepwise oxidation of the acid residue from the CM2 chondrite Murray yielded a minor phase which did not oxidize until over 1,000°C and had a $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of around +100‰ (PDB) and +90‰ (AIR) respectively, as well as a low δD . A carbonaceous phase of this nature has not been recognized either from other meteorites or from Murray by workers in the field. However, attempts to confirm Kerridge's data should renew interest in identifying and explaining the phase. Acid-insoluble residues, which contain major D enrichments for some carbonaceous

chondrites, also appear to be the D carrier in the primitive ordinary chondrites (Yang and Epstein, Caltech). The Cambridge-East Kilbride group also demonstrated that most type 3 ordinary chondrites have enrichments in D above the terrestrial range, but only the least metamorphosed show extreme enrichments. Furthermore, the D carrier in one of the least altered type 3 chondrites, Semarkona, seems to be a minor phase, which decomposes when heated to 550–650°C and has a minimum δD of over +5,500‰ (SMOW).

A controversial issue is whether ^{26}Mg excesses in Al-rich minerals are a result of *in situ* decay of ^{26}Al or whether ^{26}Mg is a relict species that was already present at the time of Solar System formation. The idea of 'fossil' ^{26}Mg was again forcefully argued by Clayton (Rice University) as being the more plausible explanation because of mixing constraints in the early solar nebula, and because of certain other isotopic anomalies such as ^{16}O and ^{50}Ti . Advocates of the *in situ* decay model point to data obtained using the ion microprobe (Huneke, Caltech), which show uniform distribution of ^{26}Mg in minerals from Allende. □

where substrate binding is accompanied by the ordering of an adjacent mobile part of the structure — a kind of flexible lid which closes over the substrate. The entropy change of such a process destabilizes the enzyme-substrate complex and thus facilitates release of products. In cases where a linked function is invoked, this ordering of a mobile chain may convey a signal to a part of the molecule which the disordered chain can touch. In the pyruvate and 2-oxoglutarate multi-enzyme complex, Gordon Roberts (National Institute of Medical Research) and Richard Perham (University of Cambridge) showed that lipoyl-lysine residues act like flexible arms to allow the transfer of substrate from the active site of one enzyme subunit to another.

Many examples of mobile molecules and many different techniques were discussed. Nucleosomes show a high degree of mobility on the nanosecond to microsecond time scale, making them a good subject for NMR study. Michael Hogan (Princeton University) has used ^{31}P magnetic resonance to study the motions of DNA in both the isolated state and in nucleosomes, supplementing these studies with fluorescence anisotropy decay measurements using DNA-binding dyes. The observed motion suggests twist or flexure of the DNA helix. Mort Bradbury (University of California, Davis) reported NMR mobility studies on the histone and non-histone proteins of nucleosomes. The unusually high mobility of these proteins seems to result from their highly charged character, which causes them to be fully or partly unfolded under almost all conditions.

Bob Williams (University of Oxford) suggested that the action of calcium-binding trigger proteins depended on motion of hydrophobic groups detectable by NMR. In muscle, motion of the 'head' part of the myosin molecule, which forms a cross-bridge linking the actin and myosin filaments, achieves relative motion between them. Measurements reported by Stefan Highsmith and Oleg Jardetzky (University of California, San Francisco) showed that mobility in parts of the myosin molecule was quenched on binding to F- or G-actin. David Thomas (University of Minneapolis) had studied the same system by EPR and spin label, and found a two-state 'relaxed' and 'attached' system with different degrees of order, modulated by calcium. The function of muscle clearly depends on molecular motion, but are these motions related to mobility on the NMR and EPR time scales? Bill Harrington (University of Baltimore) went so far as to suggest that the rotation of the myosin head was achieved by a transition between a folded and an unfolded state in the stem of heavy meromyosin which carries it.

Any dissatisfaction felt about these inconclusive speculations was dispelled by two exciting PMR papers which gave clear

Hard facts on structure: hot air about mobility

from David M. Blow

THE idea that the inherent flexibility of biomolecules relates to their function received another airing at the CIBA Foundation Meeting* on March 2–4, 1982. Improvements of technique now make available a wide portfolio of techniques for the measurement of mobility in macromolecules. The most important is NMR, which allows separate relaxation times to be measured for every line in the spectrum. Almost any other spectroscopic technique, from fluorescence to EPR, can give highly specific information about the mobility of a chromophore or a spin label. Time-resolved X-ray diffraction, which in principle can give a 'flash' picture of a structure any time after triggering an event, is still in its infancy. But X-ray crystallography can measure the time-averaged motion of individual atoms, so long as the motion is not too large, and can delineate domains of correlated motion.

Computational capacity has increased to the point where all the atomic interactions for a small protein can be simulated, allowing the dynamics of the molecule to be calculated over a period of a few picoseconds. The achievable time span is

far too short to cover a significant chemical event, but the results do appear realistic so far as they go. Martin Karplus (Harvard University) has used simulations to measure correlation times of a few picoseconds between atomic motions, and has estimated the apparent viscosity of the protein fluid in which an individual atom moves. People speak of analysing the normal modes of vibration of a protein molecule, though nobody has done so yet.

What has all this to do with the biological function of a molecule? Fred Richards (Yale University), stern but perceptive chairman of this rather unruly symposium, stressed this question in his introduction. Is mobility essential or incidental to biological action? What experiments and theories can be devised to establish a relation between them? He got few answers.

The most thoughtful came from Greg Petsko (MIT) who stressed that in many cases, access to an active site, and release of product, would be greatly hindered, if not prevented, if the adjacent structure were rigid. He cited triose phosphate isomerase and pancreatic ribonuclease as examples

*The proceedings of the meeting will be published by Pitman Books, London, in winter 1982 under the title *Mobility and function in proteins and Nucleic acids* (Ciba Foundation symposium 93).

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and definite results, using the nuclear Overhauser effect (NOE) to detect dipolar interactions between protons which are close together. If the relative populations of the nuclear spin energy levels of one proton are perturbed — for example, by saturating its resonance frequency — the dipolar interactions transmit the perturbation to adjacent protons, resulting in an altered resonance intensity. Pulses may be specifically shaped to avoid exciting the H_2O resonance. Redfield has pioneered the use of this technique in the analysis of tRNA spectra, and has been able to assign resonance lines in the D stem of tRNA^{Asp}, and also in reverse-Hoogsteen base pairs (*Nucleic Acids Res.* 9, 7073; 1981).

Brian Reid (University of Seattle) has applied this technique to the aromatic NH band, which contains just one resonance for each base pair. When such a resonance is perturbed, either one or two other NH resonances show up by NOE. These are resonances in adjacent stacked base pairs. An example is shown in the figure. In this way Reid has been able to survey all the stacked base pairs, and to assign all the corresponding 28 ring NH resonances, in tRNA^{Tyr}.

Kurt Wüthrich (University of Zurich) is tackling with Richard Ernst the more ambitious problem of the basic pancreatic trypsin inhibitor. Instead of saturating specific resonance lines, he obtains a complete spectrum by irradiating with a specific pulse at every frequency in the proton resonance band. This gives a complete two-dimensional spectrum. By using different regimes of excitation and observation separate two-dimensional spectra are obtained by correlated spectroscopy, spin-echo correlated spectroscopy and NOE spectroscopy. (The

engaging acronyms COSY and SECSY were coined for the first two, the last having a more nasal connotation.) COSY has enabled NH and C¹³H resonances in the same amino acid to be correlated, while NOESY allows the identification of β -structure interactions by the proximity of C¹³H and NH protons in adjacent residues (*J. molec. Biol.* 155, 311; 1982). By further development of these methods, almost

every resonance in glucagon and in the pancreatic trypsin inhibitor has been assigned. Individual changes of internal mobility can be observed as pressure and temperature are changed.

The claim that a three-dimensional protein structure may, in the near future, be totally determined from NMR data is beginning to look more realistic, if only for a very small, rigid protein molecule. □

Do stepping stones guide axon growth?

from Hilary J. Anderson

In a recent issue of *Nature* (June 3, p.404), Ho and Goodman present data supporting the idea that peripheral pioneer neurones in the grasshopper are guided to their destinations by a series of cellular 'stepping stones'. The 'pioneer' neurones are of vital significance because they establish the basic pathways and tracts within the nervous system that act as guides for neurones that develop later. The term was first used by Ross Harrison, in his now classic studies of frog embryos, to describe the neurones that grow out and establish connections very early in development when the distance to their target regions is very small. Even though the animal subsequently grows and changes form and the pioneer tracts twist and lengthen they continue to provide the guides that allow other neurones to reach their appropriate destinations.

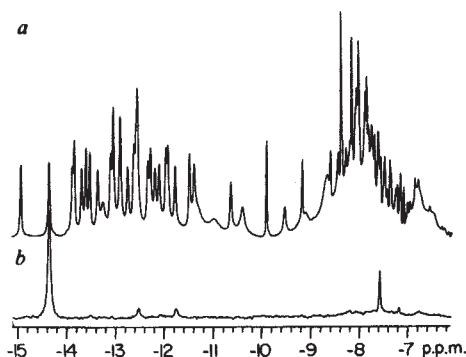
Several mechanisms of pioneer axon guidance — mechanical channeling, basement membrane markers and cellular landmarks — have been suggested and to distinguish between them it is necessary to study the behaviour of growing neurones in some detail. The tip of a growing axon is expanded into a growth cone from whose leading edge extend numerous filopodia which are 0.1–0.2 μ m in diameter and many micrometres in length and filled with actin filaments. The growth cone makes 'searching' movements over its substrate, extending and retracting its filopodia, and probably plays a fundamental part in the interactions with the environment that direct and control neurone growth. Harrison proposed that the pioneers were guided by the configurations of tissues in their environment, grooves and spaces in the embryonic tissue providing "paths of predilection" along which the growth cones progress. This idea has been supported by several recent studies using electron microscopy which show that developing or regenerating axons often grow through preformed channels or spaces (see *Nature, News & Views* 283, 428; 1980). In other instances, however, pioneers grow over regions seemingly devoid of obvious physical regularities, suggesting that there are other factors that direct pioneering axon growth.

Pioneering axon growth in the central and peripheral nervous systems of insect embryos has proved particularly amenable to experimental study. The central pioneers that will be discussed below are those that establish the primary longitudinal tracts of each ganglion in the central nervous system (CNS) (see ref.2 and *News & Views* 293, 510; 1981). Ho and Goodman studied the peripheral pioneers which establish the peripheral nerves linking the CNS and the appendages (see also refs 3–6). These neurones have been observed as they grow in the living embryo, and examined by electron microscopy, by staining with intracellularly injected fluorescent dyes and by staining with particular antibodies. In this way several different aspects of their behaviour have been successfully described.

In their recent paper Ho and Goodman establish the important point that the pioneers are not a special class of cell but may be motor neurones, interneurones, or sensory neurones. Furthermore, the pioneers themselves may grow along other pioneers for short distances during growth. It thus seems likely that the mechanisms underlying pioneering axon growth are common to all growing neurones.

Electron microscopy has shown that a basement membrane lies over the neuroectodermal layer from which the CNS develops². The central pioneer growth cones always track along this basement membrane and do so at a consistent location and in a consistent direction to form a characteristic tract². Similarly, in the periphery, where the embryonic appendages are hollow epithelial buds, the pioneer growth cones grow only over the inner surface of this epithelium^{3–5} and do so only at certain locations to form characteristic pathways^{3–5}. It has been suggested that cues within the basement membrane might provide directional information for growth cone navigation². Such cues would be imposed on the basement membrane by the underlying sheet of cells and could take

a, The aromatic NH region of the NMR spectrum of tRNA^{Val} (*E.coli*). The imino NH proton resonances lie between -9 and -15 p.p.m. In this region only the resonances whose exchange is slowed down by hydrogen bonding appear. Each represents an NH in one hydrogen bond of a base pair. b, Saturation of the resonance at -14.4 p.p.m. leads, by the NOE, to a signal at -7.6 p.p.m. from a non-hydrogen-bonded resonance in the same base pair, and also to two signals at -11.7 and -12.5 p.p.m. from adjacent hydrogen-bonded imino groups. These two signals come from base pairs stacked on either side of the base pair whose resonance is saturated. (Courtesy of B.R. Reid, University of Washington.)



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the form of a simple indicator of longitudinal polarity, or a continuous gradient of information or a series of discrete markers.

Other observations have suggested the cellular landmark hypothesis favoured by Ho and Goodman. Before the peripheral pioneers axons appear, glia-like cells are found in the lumen of the appendage and in contact with the inner surface of the epithelium. These cells are found at regular intervals along the presumptive path of the pioneers³⁻⁵ and could provide a series of stepping stones between the tip of the appendage and its base³. Later the glia ensheath the pioneers to form a proper nerve bundle. Ho and Goodman observed several other conspicuously located cells which could act as landmarks for the pioneers. Furthermore, the monoclonal antibody which they used for many of their observations stains growth cone filopodia very clearly and shows that they extend for some 50 μm . Thus, at no time need an axon be navigating without being in 'filopodial grasp' of a landmark cell.

The three proposed substrates for guiding pioneer axon growth now need to be tested by experimental manipulation, such as selective ablation of putative landmark cells. Ultimately these studies must lead on the one hand to an investigation of the molecular mechanisms underlying the preferential growth of pioneers over their chosen substrates and on the other hand to a study of the principles of spatial organization which generate these substrates at their appropriate locations — the fundamental unanswered question of developmental biology. $\square$

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Discovery of pre-galactic lithium

from Bernard Pagel

THE reality of the hot big bang is attested by galaxy redshifts, the 2.9K microwave background and evidence for evolution in the statistical properties of distant quasars. Indeed, if grand unified theories fulfill all their promises, then such basic facts as the existence of matter (rather than antimatter) and electric charge can also be taken as consequences of the big bang. These fundamental effects were imprinted on the Universe less than 10^{-35} seconds after it was born, while the last scattering of the microwave background took place about a million years later when protons combined with electrons and space became transparent. Galaxies and stars were presumably formed later still.

In an intermediate phase, when the Universe was about one second old and its temperature around 10^{10}K , neutrinos decoupled leaving an excess of protons over neutrons. During the next two minutes or so these combined in nuclear reactions to make deuterium, helium-3, helium-4 and lithium-7, production of heavier elements being aborted by the absence of stable nuclei at mass numbers 5 and 8. The resulting pre-galactic composition of cosmic matter, consisting chiefly of hydrogen and helium-4 in proportions of about 3:1 by mass, has since been modified by cycling of some of the material through stars. This has affected material in our neighbourhood by adding a mass fraction of 1 or 2 per cent of carbon and heavier elements and a similar amount of additional helium-4. Deuterium, on the other hand, is totally destroyed in matter cycled through stars, and helium-3 and lithium-7 can be both created and destroyed, so that the net effect of stellar evolution on these two species is rather uncertain.

A remarkable claim by Monique and Francois Spite, of the Meudon Observa-

near Paris, that something at least very close to the pregalactic lithium abundance can be measured directly (see this issue of *Nature*, p.483), may now provide additional constraints on this view of the early Universe.

The pregalactic composition of cosmic matter depends on a number of parameters of cosmology and particle physics, notably the ratio of baryons to photons (or, equivalently, the present mean density of ordinary baryonic matter) and the number of lepton flavours, together with the net charge number and various lepton numbers that are believed to be zero or negligibly small. As a result, the determination of pregalactic abundance provides interesting constraints on these parameters and tests the consistency of the entire picture. For example, the presence of observable deuterium in the interstellar medium implies that the Universe cannot contain a sufficient density of ordinary matter to 'close' it, that is, eventually to halt the expansion by gravity, although it could be closed by non-baryonic matter such as massive neutrinos, if these exist. A still more severe upper limit to the density is provided by the observation that the mass-fraction of helium in emission-line galaxies of low oxygen abundance (in which the gas has accordingly undergone little recycling through stars) is below 25 per cent, which implies that the baryon/photon ratio (resulting from the primordial excess of matter over anti-matter) is below 4×10^{10} or $\Omega_b < 0.03$ (assuming three neutrino flavours and a Hubble constant of $75 \text{ km s}^{-1} \text{ Mpc}^{-1}$) where Ω_b is the fraction of the closure density supplied by ordinary baryonic matter. If, however, the density were less than about $\frac{1}{2}$ of this upper limit, corresponding to a helium abundance of under 23 per cent, then the expected pregalactic abundances of deuterium and helium-3 would become embarrassingly large. Thus a really accurate determination of the pregalactic helium abundance would provide a very severe test of the consistency of canonical big bang models, if only it could be carried out. Unfortunately, the prospects of finding the pregalactic helium abundance to better than 5 per cent are not very good.

What about lithium in all this? The 'cosmic' mass fraction of lithium-7, deduced from meteorites, is 5×10^{-9} and a similar amount is found in the atmospheres of the youngest stars. In older stars, however, and notably in the Sun itself, lithium is depleted because the surface layers are mixed in the course of time with

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100 years ago

THE mines opened a short time since in China in the province of Chihli, with the special support and patronage of Li Hung Chang, have recently become the subject of much adventitious interest in Europe. It was believed that, with sufficient transport, one thousand tons a day could be raised for many years from the present pits, while it was said that fifty collieries of an equal size to the present one could be opened in or near Kaiping. The information, therefore, telegraphed by Reuter's agent in Shanghai that the further working of the mines had been peremptorily stopped by the Government, came with a shock to many interested in progress in China. It was stated that a censor in a memorial to the throne complained that the long galleries in the mines, and the smoke of the foreign machinery, disturbed the earth dragon, who in his turn disturbed the spirit of the Empress, who died some months ago, and

who was buried about a hundred miles off. The irate spirit of the departed lady promptly took vengeance by afflicting the denizens of the palace in Peking with measles. The latter were, the censor is reported to have said, distinctly traceable to the Kaiping mines, which interfered with the *feng-shui*. The conclusion was obvious: the mines must be stopped. Such was the story told by the Tientsin correspondent of a Shanghai newspaper. The latest information from the East enables us to say that the mines are still working as usual, and there is not the slightest evidence that there is or has been any intention of interfering with them. It is even denied that such a memorial as that mentioned above has had any existence except in the imagination of a *gobemouche* at Tientsin. However this may be, it must be confessed that the petition has a Chinese ring about it, and that the method of argument is one sufficiently familiar to readers of the *Peking Gazette*.

From *Nature* **26**, 136, 8 June 1882.

deeper layers at a temperature of 2×10^6 K where lithium nuclei are destroyed. The degree of depletion in ordinary stars increases with age and with diminishing surface temperature because cooler stars have deeper outer convection zones. A few highly evolved stars make fresh lithium, however, and yet more may be generated by cosmic-ray spallation in the interstellar medium, so extrapolation from present-day to pregalactic lithium abundance is a hazardous business.

What Monique and François Spite have done is to estimate the lithium abundance of a sample of extreme subdwarfs. These are old stars with extreme metal deficiency, typically a factor of 100 down on the Sun for carbon and heavier elements, so that the material from which they were formed could have undergone little previous cycling through stars. Out of the five stars of differing surface temperature that they studied, the four hottest turned out to have one-tenth of the present-day lithium abundance — a strikingly large amount in view of the overall metal deficiency — and only in the coolest star was it undetectable, presumably because of depletion through convective mixing with deeper layers. The authors argue that the constancy of lithium

abundance in the four hotter stars indicates that none of these has been significantly affected by lithium depletion. If they are right, then they have a value for the pregalactic lithium abundance; if not, then they still have an interesting lower limit.

What does this new result tell us about the big bang? Unfortunately the predicted lithium abundance is a variable and generally fairly insensitive function of baryon density and, furthermore, it is rather uncertain. For example, Olive and co-workers (*Astrophys. J.* **246**, 557; 1981) suggest an upward revision by a factor of 3 over the earlier calculations referred to by Spite and Spite; in this case the minimum possible value is minutely (25 per cent) greater than that observed, although it is striking that this value occurs at parameter values close to those deduced from helium and other light elements. A fairly conservative view of the errors (especially with regard to possible depletion) suggests that lithium in subdwarfs provides an upper limit to the cosmological baryon density that is consistent with, but less stringent than that provided by helium. What is exciting about the Spites' discovery is that lithium is there at all, and that its amount is not far from big bang predictions. □

Stable gene expression *in vitro*

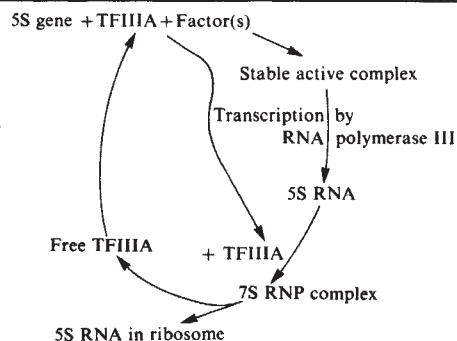
from H.R. Woodland

ONE characteristic of cell differentiation is its high degree of stability, and there is a suspicion among molecular biologists that this derives from a basic stability in the expression of genes involved in differentiation. Our understanding of the issue is unlikely to improve beyond this point, however, until systems are developed which enable active genes to be reconstructed *in vitro*. Bogenhagen, Warmington and Brown of the Carnegie Institution of Washington, together with R.G. Roeder's group at the University of Washington, may now have developed just such a system. In a recent issue of *Cell*¹, they describe the construction of 5S gene protein complexes with the properties of stable gene expression or repression that have been suggested to occur in intact cells.

As much is known about 5S gene transcription as for any other eukaryotic gene (see Korn's recent review in *Nature*²). 5S genes encode 5S RNA, a component of all ribosomes except those of animal mitochondria. Like tRNA and a few other small RNAs, 5S RNA is produced by RNA polymerase III, a different enzyme from that which makes mRNA. The unusual characteristics of this polymerase mean that the 5S genes differ in structure from genes encoding mRNA. Surprisingly, the only part of the 5S gene actually essential for its transcription is residues 50–83 of the

120 nucleotides that are transcribed. This region binds a 40,000 molecular weight protein, called TFIIIA by Roeder's group³, which produces accurate transcription of the gene. TFIIIA also binds to 5S RNA, forming a 7S ribonucleoprotein (RNP) particle. This may provide a means of transcriptional regulation whereby the free protein stimulates 5S RNA synthesis, but is removed by the product of this synthesis (see the figure). A last important feature of 5S RNA, at least in *Xenopus laevis*, is that oocytes and somatic cells contain 5S RNAs of different sequence. The main oocyte 5S RNA is encoded by 20,000 genes called 5S *ooc*, and that in somatic cells by a separate set of 400 genes called 5S *som*.

Why do somatic cells contain different 5S RNAs? Selective transcription of the 5S *som* genes seems the cause. In oocytes, some 5S *som* RNA is made, perhaps in proportion to the small number of 5S *som* genes, but the RNA seems not to enter ribosomes and is degraded⁴. Free 5S RNA is known to have a short lifetime in both oocytes and somatic cells^{6–8}. Unfortunately, the role of degradation in regulating 5S gene expression has never



Possible scheme of 5S RNA synthesis. In a very young amphibian oocyte, 5S RNA is made in great excess of other ribosomal components and enters a 42S RNP particle, instead of a ribosome. As the 42S RNP particle does not contain TFIIIA¹⁰, the latter is released and can stimulate further 5S synthesis, just as in the normal cycle shown here.

been accurately quantified in any system.

The latest experiments from Brown's laboratory¹ use cell-free systems made from either oocytes or somatic cells. If 5S DNAs of different types are added simultaneously to a cell-free system they compete for transcription⁹. It has now been shown, however, that if two DNAs are added at different times, the first is transcribed in preference to the second¹. Indeed, if enough of the first DNA is added subsequent DNAs are not transcribed at all, no matter how much is added. The first DNA seems to form a very stable, transcribable complex within a minute or two. This is not an actual transcription complex, since maximal transcription is established much more slowly; thus agents other than the polymerase, but necessary for polymerase binding, must be involved in formation of the complex. It might be guessed that TFIIIA is one such agent, but although 5S DNA may be bound to TFIIIA, this alone does not confer favoured transcription status upon it.

Bogenhagen *et al.*¹ were also able to make stable inactive 5S gene complexes. Using a transcription system depleted of TFIIIA by antibody treatment, they showed that 5S DNA does not form the active complex unless further TFIIIA is added. However, if histones were added instead of TFIIIA, a different complex was formed which could not then be reactivated with TFIIIA. The possibility that this complex is an artefact is unlikely, since the transcription of tRNA genes is unaffected by added histones (tRNA genes do not require TFIIIA for transcription).

Bogenhagen *et al.*¹ speculate that the stable complexes they have constructed *in vitro* have similar counterparts in cell-free systems. This remains to be proved. At least natural 5S DNA protein complexes show similar stability to those made in cell-free systems — chromatin added from somatic cells or oocytes to the same systems used to transcribe the pure 5S DNAs continues to express only those genes which were active in the intact cells from which it was derived. This emphasizes that the cell-

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free systems do not reprogramme chromatin, nor indeed do oocyte or somatic cell-derived systems discriminate significantly between 5S *ooc* and 5S *som* genes. Why then are these genes expressed differently in oocytes and somatic cells? Bogenhagen *et al.*¹ suggest that the activity of the 5S genes is determined by events during chromatin construction at DNA replication which establish an active or inactive structure that persists throughout the ensuing cell cycle. The two states could be propagated through the next DNA replication unless hypothetical factors are present which change the state of the gene. This hypothesis is consistent with many observations suggesting that DNA

synthesis is necessary for changes in the differentiated phenotype of cells. It does not fit, however, with Korn and Gurdon's⁵ observation that the oocytes of certain *Xenopus* females can activate the oocyte

genes of somatic nuclei in the absence of DNA replication.

Bogenhagen *et al.*¹ also suggest that what goes for 5S genes might also apply to genes involved in cell differentiation. The dissimilarity of the transcription of the two sorts of genes by RNA polymerase III and RNA polymerase II respectively does not encourage heavy bets on this possibility. Yet advances in this area certainly depend heavily on developing cell-free systems which accurately reproduce the gene regulation of intact cells. There is no doubt that the new 5S systems show exciting possibilities in this direction, even if they do not yet solve the problems of cell differentiation. □

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Leg 84 of the Deep Sea Drilling Project

Subduction without accretion: Middle America Trench off Guatemala

from Jean Aubouin*, Roland Von Huenet†, Miriam Baltuck‡, Robert Arnott§, Jacques Bourgois*, Mark Filewicz, Keith Kvenvolden†, Barry Leinert**, Tom McDonald‡‡, Kristin McDougall‡‡, Yujiro Ogawa§§, Elliot Taylor‡‡ and Barbara Winsborough

PRESENTED below are the first results from the recent visit of the drilling vessel *Glomar Challenger* to the southern Guatemalan segment of the Middle America Trench (Leg 84 of the Deep Sea Drilling Project/International Phase of Ocean Drilling, January–February 1982). The area, shown in Fig. 1, had been selected for investigation because of several interesting problems that had been posed by earlier visits to the region. In 1979, Legs 66 and 67 had produced contrasting results from the two halves of the Middle America Trench divided by the Tehuantepec Ridge — on Leg 66 ocean sediment was found to be accreting against the landward slope of the trench, while on Leg 67, off Guatemala, no accretion was found. Leg 67 results come mainly from one site (494) on the landward slope of the trench where basement was reached³; drilling at three other sites were abandoned when gas hydrates were

unexpectedly recovered.

Seabeam⁴ and deep tow instruments were also used to survey the area examined by Leg 67. The original fabric of the Cocos Plate at the East Pacific Rise is delineated by magnetic anomalies which trend 20° from the direction of the trench axis. As the Cocos Plate is flexed into the trench, it breaks into oblique extensional ridges and troughs paralleling the original fabric of the Cocos Plate, and the surface expression is one of collapse into the subduction zone.

On the landward slope of the trench the structural picture is less clear. Palaeogene

and Cretaceous rocks were recovered at its base, suggesting non-accretion off Guatemala and showing that a tectonic history exists below the Neogene slope sediments. More definite conclusions could not be drawn because only one penetration had been made below the Neogene slope deposits. The recovery of visible gas hydrate made further drilling unsafe because the base of the gas-hydrated sediment had, at the time, not been defined in seismic records and it was feared that free gas might be trapped at the base of an impermeable hydrated section.

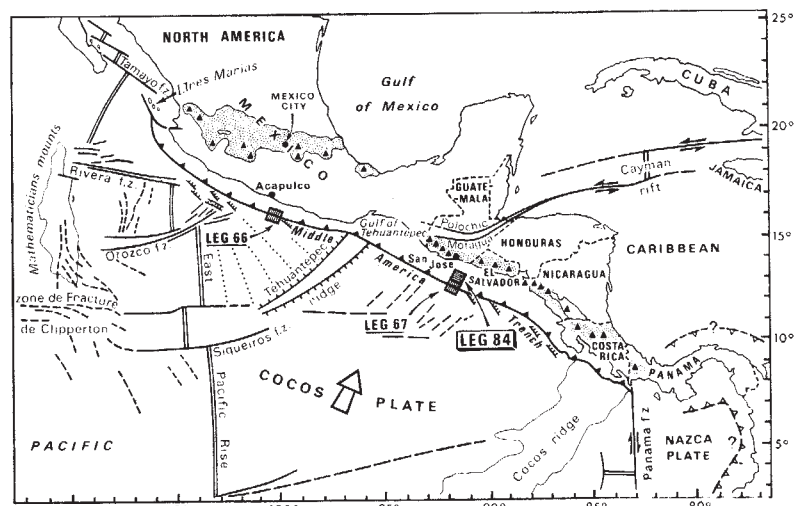


Fig. 1 Sketch map of Middle America Trench.

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Leg 84 was recommended by the JOIDES advisory panels specifically to examine the tectonic and gas hydrate problems. Local base of gas hydrate reflections were found in a re-examination of predrilling site survey data and after enhancement and combination with refined temperature gradient information, the depth of the base of gas hydrate was established. Basements high above the base of gas hydrate were selected for drilling.

At the penetration sites (Fig. 2) basement was found to consist of ophiolitic rock covered by sediments of early Eocene (sites 569, 570), late Oligocene–early Miocene overlying late Cretaceous (567) and late Miocene (566) age. No age progression (younger rocks at the base to older at the top of the slope), as admitted over the supposed accreted complex off Mexico where only Neogene deposits were drilled, was found. The sites are distributed transverse to the margin and are laterally separated by as much as 100 km. Peridotite (harzburgite), gabbro, dolorite, basalt and much serpentine in various stages of decomposition were recovered. These rocks were of a metamorphic grade from low-temperature to greenschist facies and were tectonically juxtaposed without particular order. The Guatemalan margin is therefore underlain by tectonically disrupted ophiolitic rock.

The ophiolitic rock was tectonized and the tectonic complex was emplaced before the present arc-trench tectonic system. Clastics of ophiolitic rock were included in the unaltered slope sediment, particularly at the base of the slope where debris flows contain large blocks of serpentinized peridotite. Other studies⁵⁻⁷ suggest the tectonic history includes three major periods:

(1) Ultramafic rock is thrust over the Jurassic–Cretaceous Nicoya complex⁶ and is in turn overlain unconformably by Campanian sediments, as is evident on the Santa Elena Peninsula⁸. Initial tectonism of the Guatemalan ophiolitic rock could have started at this time, and the Cretaceous sediment at the base of the slope could correspond to the Campanian sediment cover.

(2) A strong Palaeocene uplift, documented in the Petrel well⁹, may immediately pre-date the early Eocene sediment recovered at two sites.

(3) A strong Oligo–Miocene faulting left a sharp angular unconformity observed in seismic records across the edge of the shelf; the first thick hemipelagic slope mudstone deposits of the present tectonic system developed during and after this event.

There is nothing to indicate development of the present arc-trench system until the late Oligocene when the first substantial layers of volcanic ash are seen in the slope cover. The ophiolitic rock of the Guatemalan margin was certainly emplaced in pre-Eocene time and appears to be an extension of the continent of Central America. At the base of the slope

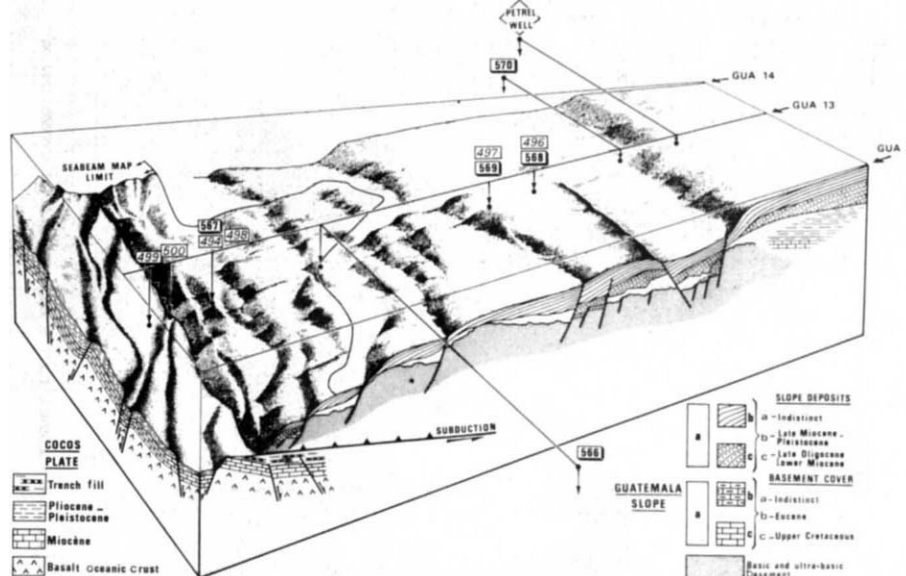


Fig. 2 Block diagram showing the convergent zone between Cocos Plate and Caribbean Plate in Leg 84 area. The morphology is reconstituted from Jean Charcot seabeam data and GUA 2, GUA 13 and GUA 14 seismic profiles of Texas University.

(Site 567) we came close to penetrating through the upper plate into the subduction zone. Here, Miocene to present oceanic and trench sediments are being subducted beneath the pre-Campanian ophiolitic rock at the front of the margin. Drilling ended when the hole collapsed, and the drill string became stuck. Collapse was accompanied by overpressured pore fluids — overpressure had also been observed at sites in top, mid and lower slope. Seismic records across the mid and upper slope sites show few compressional faults or folds in the slope sediment where the overpressures were observed. In fact, the most obvious structures on the transect are normal faults at the base of the slope. Elevated pore pressures are to be expected across the subduction zone, and such pore pressures help to explain the degree of decoupling required to subduct soft sediment beneath a mass of hard ophiolitic rock. High pore pressure appears to be very common in the Guatemalan convergent margin and occurs even in slope deposits that show few structures.

Many local unconformities and hiatuses were detected in sites drilled in slope deposits on the Guatemalan margin and indicate that as one depocentre is rapidly accumulating sediment, another may have lost its feeder channel and temporarily be in an interchannel or erosional area. Hollows and topographical flats from initial faulting of the surface of the ophiolitic mass are sites of ponded basins or early Miocene prograding sediment bodies. The sediment in these sequences locally contains more sand than is found in the sediment ponded in the trench axis. Sand was recovered in the channel areas, demonstrating again that the channels transport sand directly to the trench. The presence of a large amount of sand and rapid sediment accumulation is not a lithological criterion by which to distinguish between trench and slope

deposits off Guatemala.

Slope deposits off Guatemala are now classic sites for gas hydrate¹⁰ which was detected at all sites, except 567, and was recovered many times at 568, 570. The best recovery was at site 570 where a massive 3 m thick gas hydrate was cored, and samples were preserved in special pressure vessels for onshore studies. The gas hydrate was recognized: (1) visually, (2) by volumes of gas released during hydrate decomposition that greatly exceeded the volume of gas in gas-saturated water, (3) by composition — the hydrocarbon gases combined no molecules larger than isobutane, (4) by the decrease in chlorinity and salinity characteristic of the freshwater composition of gas hydrates, and (5) by sonic and density logs showing high velocities and corresponding low densities. These are the first demonstrated log responses of cored gas hydrates in oceanic sediment and provide the first measurements of their *in situ* sonic velocity and density.

Gas hydrates were found dispersed in very fine-grained sediments; associated with porous, coarse-grained sediments; occupying fractures; and as a massive unit. Geophysical records off Guatemala do not show strata corresponding to the massive gas hydrate or any other gas-hydrated horizon; only the base of gas hydrate reflection is laterally continuous, and this reflection was observed at many places on the slope, namely at one of the sites occupied (568).

Inorganic geochemical studies on Leg 84 confirm the proposed relationship between low values of salinity and chlorinity and the occurrence of gas hydrates. In all cases where gas hydrates were observed on Leg 84, values of salinity and chlorinity were lower than in sediments where gas hydrates were absent.

Most gas hydrates found were composed of gases from microbial and early diagenetic processes. However, at two sites

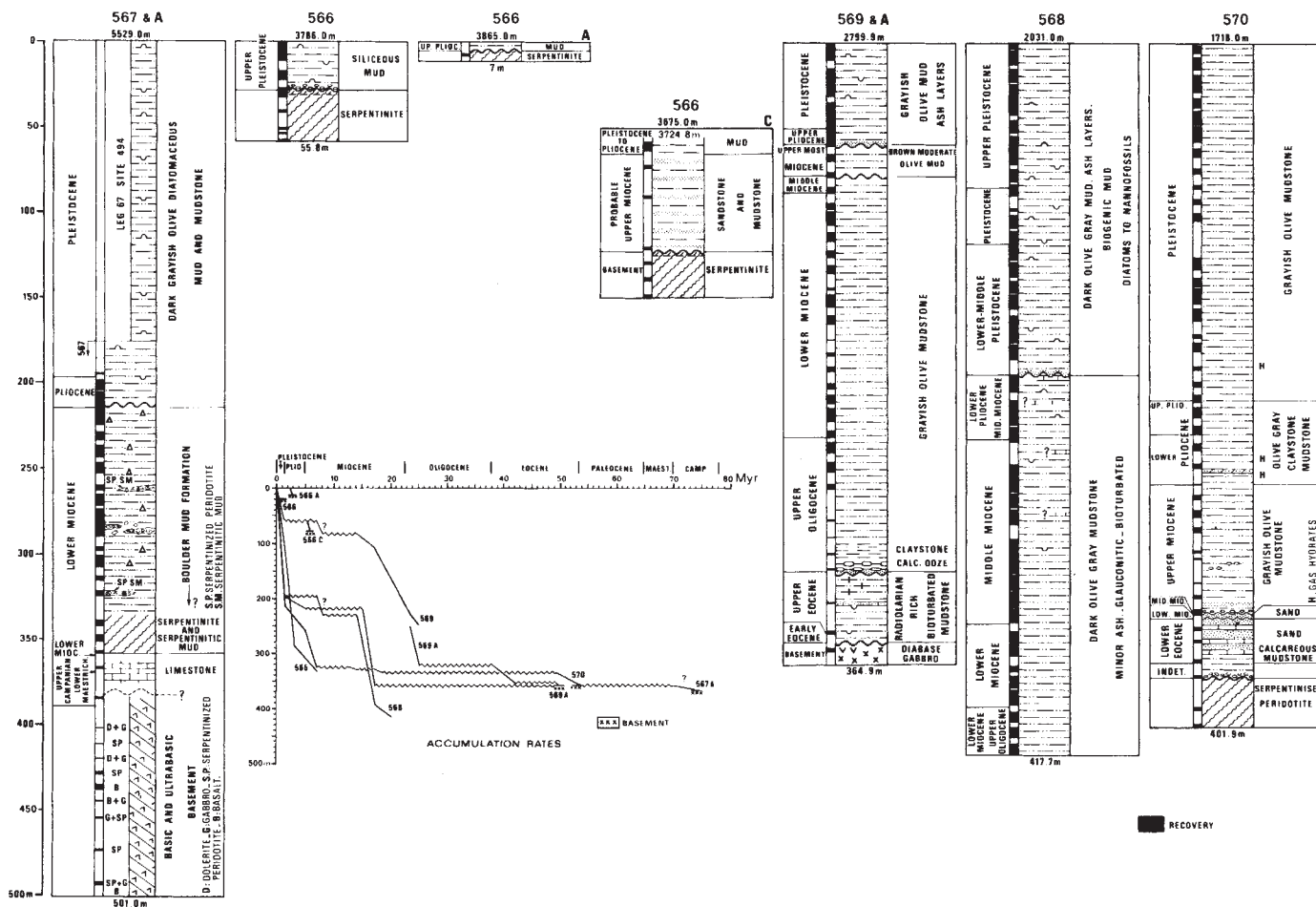


Fig. 3 Simplified stratigraphical columns summarizing the lithostratigraphy, biostratigraphy and recovery of Leg 84 drill sites.

(566 and 570) the gases found associated with serpentinite were probably a product of the thermal breakdown of organic matter because they contained very high concentrations of hydrocarbon gases, particularly of ethane relative to methane, and hydrocarbons at least as large as hexane and heptane.

Conclusions

(1) The DSDP/IPOD Guatemalan transect across the Middle America trench has defined a non-accretionary convergent margin; Neogene subduction has not resulted in the development of an accreted complex; rather, a mass of tectonized ophiolitic rock now faces the subducting Cocos Plate (Fig.3). The ophiolitic rock was emplaced before Eocene time in a previous tectonic system: at the trench the Cocos Plate is of Miocene age.

(2) A horst and graben topography gives the Cocos Plate the appearance of collapsing into the trench, and the adjacent base of the Guatemalan margin is also normally faulted. However, given the 10 cm yr^{-1} plate convergence rate, this extensional topography must be surficial and secondary. The topography of extension in a fundamentally compressional environment can be explained on the seaward slope of the trench by flexure of the Cocos Plate; on the landward

slope of the trench it may be explained by a high degree of decoupling across the subduction zone. Decoupling is required to subduct sediment on the lower Cocos Plate beneath the ophiolitic upper plate rather than accreting it. A classical mechanism for such decoupling is overpressured pore fluid such as that measured only a short distance above the Middle America subduction zone. Elevated pore pressure was measured not only in the vicinity of the subduction zone but also in slope sediment across the transect, indicating a general overpressure condition throughout much of the margin.

(3) Beneath the cover of slope sediment, the basement of the Guatemalan margin is an extension of the Central American continent.

(4) The major results of Leg 84 were

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achieved by drilling below the present slope sediment and into basement. Accretionary complexes may be defined by their geometry in seismic reflection records, but results from most of the convergent margins drilled during the IPOD programme indicate that the accretion observed in seismic records may belong to previous tectonic systems; accretionary periods may alternate with non-accretionary periods. Thus, a seismic record alone may be insufficient to establish the tectonic history or the present tectonic system in a convergent margin. Unless the accretionary complex is sampled, its time of emplacement and relation to the present tectonic system is uncertain.

(5) Gas hydrates are probably dispersed throughout many marine sediment sequences that lie in the zone of gas hydrate stability; they can be detected by seismic techniques when bottom-simulating reflectors appear on seismic records. The occurrence of visible gas hydrates in cores is in part a function of effective porosity and perhaps permeability. Off Guatemala gas hydrate is found finely dispersed in mudstone, as a solid in fractures, in the pore space of sandy ash layers, and in massive form, filling voids of unknown origin.

REVIEW ARTICLE

Radar research on thunderstorms and lightning

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Weather radars can resolve many phenomena related to thunderstorms. Echoes from clear air, water and ice condensate, and ionized lightning channels can all be identified. Radial velocity data from two Doppler-radars are synthesized to show a storm mesocyclone with updraft speeds as large as 40 m s^{-1} , moist boundary layer air flowing into the storm, and outflow into the upper troposphere. Maximum wind speeds in tornadoes can be remotely measured using Doppler spectra obtained with a high PRF radar. Doppler radars can detect thunderstorm gusts even when they are in the clear air many tens of kilometres away from the storm, and radar detection of the ionized channels caused by lightning opens new avenues into thunderstorm research.

EVERY year, starting in early spring in the southern states of the USA and ending in mid-summer in the southern provinces of Canada, severe local storms sweep across the Great Plains of North America bringing tornadoes, hail, damaging gusts, and lightning, as well as beneficial rains and cooling outflows. These storms, each a few tens of kilometres in diameter, ingest warm moist air trapped in the first kilometre or two of the atmosphere, convert moisture to rain and sometimes hail, and vent air heated by condensation into the cold upper (9–13 km) troposphere.

Researchers have been observing stormy weather over the past few decades with various instruments. One of the most significant of these is the Doppler weather radar. Radar is the only remote sensing instrument able to expose the internal structure of thunderstorms veiled by clouds that give little evidence of the enclosed hazards. Atmospheric research with radars in the USA is principally supported by: (1) the National Science Foundation, which supports universities directly and through the National Center for Atmospheric Research, (2) the National Oceanic and Atmospheric Administration (NOAA), which maintains and operates a network of weather radars for storm hazard warnings and which contains the Environmental Research Laboratories that support weather radar research having potential operational application, and (3) the Department of Defense, which supports weather radar research through its Air Force Geophysics Laboratory. The French¹ have a small but well organized Doppler weather radar research programme, and the Soviets² have long used Doppler radars in weather research. The Canadians^{3,4} have a strong research programme to determine precipitation characteristics (such as shape, size, and ice or water) using radars with polarization diversity and dual wavelengths. Recently, the British⁵ have shown how dual polarization radar can better identify precipitation type and improve rainfall rate estimates. Research on rainfall and hailfall accumulations, the foundation for many years of weather radar research, continues in the USA^{6,7}, Switzerland⁸, Japan⁹, and other countries^{10–13}. Recently, aeronomists in the USA^{14,15} and Germany¹⁶ have focused large area (for example, $10,000 \text{ m}^2$), long-wavelength (6 m) Doppler radar antennas into the troposphere to study the air motions in cloudless skies. Researchers have also used short-wavelength (10 cm) Doppler weather radars to map winds in the clear air prestorm environment and have obtained results suggesting improvements in prediction of storm location and severity^{17,18}.

Although lightning is necessary in thunderstorms, only recently have there been coordinated studies to undertake simultaneous measurements of the storm's electrical properties, its precipitation intensity, and its wind fields. One such coordinated effort has been the Thunderstorm Research International Program¹⁹ in which experiments have been conducted in Florida

and New Mexico in the USA. Initial results of these and other experiments show correlations between lightning and winds inside the storm^{20,21}. Not only does lightning seem to be linked to storm dynamics in the highly convective spring and summer thunderstorms, but it also seems related to the dynamics in weaker winter storms. The recent observations by Brook *et al.*²² show a correlation between the occurrence of lightning that transfers positive charge to earth and the vertical shear of horizontal wind in the winter thunderstorms along the Hokuriku coast of Japan.

There are many research organizations investigating various aspects of weather phenomena, but at NOAA's National Severe Storms Laboratory (NSSL), located in Oklahoma in the heart of the Great Plains, observations and research are focused on the interactions between storm electricity, precipitation, and wind in severe thunderstorms using: (1) two 10-cm wavelength pulsed Doppler weather radars powerful enough to sense the evolving wind fields in the prestorm environment; (2) the USA's tallest meteorologically instrumented tower; (3) a network of surface-based electrical and meteorological sensors; (4) a unique passive network of radio receivers to map lightning inside clouds and to locate the strike point of cloud-to-ground flashes; and (5) a system to guide instrumented aircraft in their penetration of the intense and turbulent storms.

The continued study of storm electricity, precipitation, and dynamics with recently developed remote sensing instruments could improve our understanding of how thunderstorms organize themselves. Improved predictions and warnings of hazards should result. We now summarize aspects of the use of radar to observe remotely the hazardous phenomena of thunderstorms.

Doppler weather radar

As its beam sweeps through the storm, the weather radar maps the distribution of precipitation by measuring reflectivity factor Z (the product of the number of drops per m^3 and the average sixth power of their diameter expressed in mm (ref. 23)) and generates a three-dimensional 'photograph' of reflectivity fields, typically in 3–5 min. Transmitted radar pulses also reflect from ionized paths created by lightning within the radar beam. Furthermore, radar can detect echoes returned from the small irregularities of the air's temperature and humidity in the optically clear atmosphere²⁴. Irregularities of centimetre size and temperature variations as small as 10^{-3}°C produce reflectivity detectable by radar, although invisible to the naked eye. However, the eye can see the scintillation of stars caused by these temperature fluctuations. Echoes from such targets in the first kilometre of the clear atmosphere can be seen in Fig. 1b.

Doppler radar adds information through its ability to measure target speeds. Doviak *et al.*²⁴ have reviewed the principles of

Doppler weather radar, and we highlight those to help interpret the data we present. The motions of targets towards or away from the radar (that is, radial velocities) cause minute shifts (~ 0.01 p.p.m.) in the microwave frequency of echoes in the same manner as a passing train causes shifts in the observed pitch of its whistle. Because there are usually many millions of targets moving at different velocities, the Doppler radar actually measures a spectrum of radial speeds within its resolution volume, defined by the beamwidth and the pulse length of the transmitted radiation. The resolution volume is an inherent characteristic of the radar for zero antenna scan rate. Because echoes from several tens of transmitted pulses are required to determine the Doppler spectrum, the volume contributing to the spectrum is larger than the resolution volume²⁵ if the beam scans while receiving echoes. This larger volume is defined as the measurement volume. The width of the radial velocity distribution within the measurement volume can be $<1 \text{ m s}^{-1}$ when there is no wind shear or turbulence, or as much as 100 m s^{-1} when a tornado is contained within the volume. The average velocity v and the variance σ_v^2 of the radial velocity distribution are the first and second moments of the Doppler spectrum.

Because the Doppler radar measures only the component of target velocity in the direction of the radar, three or more radars must map Doppler speeds nearly simultaneously from different directions to synthesize the three components of velocity. However, the three components of wind are connected by the mass continuity equation so that only two spaced radars are sufficient to estimate the wind field²⁶. Because ice crystals and water drops fall through the air, estimates of their fall speeds relative to the air (terminal velocities) need to be made when these are the tracers of wind. (Note that velocities relative to the ground are fall velocities.) Terminal velocity estimates are usually based on the reflectivity of the hydrometers²⁷; larger ones have higher terminal velocities and reflectivities. Refractive index irregularities and lightning channels have zero terminal velocities.

Observations

Storm structure is well illustrated when data from a single radar are displayed on a range height indicator (RHI) that gives vertical cross-sections of Z , v , and σ_v . Figure 1a, b shows respectively, Z (scaled in decibel units, dBZ) and v , in cross-sections along a 347° azimuth, for a severe thunderstorm. Visible in the Z field (a) is an anvil of cloud particles extending to the left (SSE) at $\sim 10 \text{ km AGL}$ (above ground level). More of the anvil is evident in the velocity display (b) for which the signal-to-noise ratio (SNR) threshold has been reduced to show the motion of the weakly reflective particles, which are probably ice crystals. Storm overhang and strong reflectivity gradients below 2 km AGL on the storm's south side suggest strong inflow into the storm. If we assume that the hydrometers are liquid and have the Marshall-Palmer²⁸ exponential dropsize distribution often used by radar meteorologists, then the 20 dBZ contour near the ground corresponds to a rainfall rate $<1 \text{ mm h}^{-1}$ and $30, 40$ and 50 dBZ to $\sim 3, 10$ and 50 mm h^{-1} , respectively. Often the high reflectivity regions ($>50 \text{ dBZ}$) extending to the ground contain wet hail, which causes errors in the estimate of rainfall rate. Melting ice crystals mixed with rain in the region (melting layer) between the freezing temperatures above and warmer temperatures below can also cause a significant error in rainfall rate estimates.

The Doppler velocity field in Fig. 1b depicts motions to the right and away (positive velocity) from the radar as well as motion towards (negative velocity) the radar. The storm outflow, assisted by northwesterly winds outside the storm, carried cloud particles in the anvil causing it to grow southeastward towards the radar. In the anvil, air velocities were more negative than -27 m s^{-1} , the Nyquist limit²⁴ of the radar, so they appear as high positive values (but less than $+27 \text{ m s}^{-1}$) and are seen as patches of lightest red embedded in regions of

lightest green. The momentum of updraft air carried condensate into the lower stratosphere where negative buoyancy forced air downward and outward around the updraft. This latter motion is evident in Fig. 1b above the anvil on the storm's southern side where we see the highest radial velocities.

The velocity data in Fig. 1b above the upper edge of the boundary of the reflectivity field shown in Fig. 1a are due to antenna sidelobes receiving echoes from the high reflectivity regions when the main beam lobe is pointed above the storm (due to reception of signals from directions away from the antenna axis at angular distances larger than the beamwidth). These falsely mapped velocities do not portray the true wind there. Antenna sidelobes constitute one of the more important limitations to expanding the utility of Doppler radars. However, if there are no strong echoes at a given range in the main or sidelobes, then the Doppler radar can accurately sense the velocities in weak echo regions in the main beam at that range. For example, weakly reflecting targets drifting with the flow in the clear air boundary layer (low altitude, red region in Fig. 1b between ranges of 50 and 60 km) trace the air moving into the storm.

Although radial velocity fields from one radar exhibit many interesting storm features, they cannot unambiguously depict the wind. Figure 2a shows a horizontal cross-section of a tornadic storm that formed in central Oklahoma²⁹ on 2 May, 1979. The three components of wind were obtained from radial velocity measurements made by two radars spaced 70 km apart, a reflectivity-terminal velocity relation, and integration of the mass continuity equation using suitable boundary conditions at the ground and the cloud top³⁰. The large circulation of air seen in Fig. 2a is a thunderstorm cyclone. Because thunderstorm cyclones are much smaller than extra tropical ones or tropical cyclones, including hurricanes, these circulations are called mesocyclones. Not all thunderstorms develop mesocyclones, but when they do so, they have the potential to generate large and long-lived tornadoes as did this one. In all the cases observed, large tornadoes have been preceded by mesocyclones. Mesocyclones have diameters ranging from a few to $\sim 10 \text{ km}$ and can extend in height from near the ground to near the tropopause. Tornadoes are smaller scale vortices imbedded within the mesocyclone, and if their centres do not coincide (as in Fig. 2a), the tornado circulates around the mesocyclone centre forming a trochoidal path on the ground as the mesocyclone and tornado are translated by the moving storm³¹. Note that the small scale tornado vortex is not resolved in Fig. 2a.

The inflow of air from the south-east in the first 2 km AGL , and updrafts as large as 40 m s^{-1} can be seen in Fig. 2b. Inflow air lacking precipitation targets is also detected by radar as shown in Fig. 1b. Large gradients of reflectivity mark the region of inbound air, and strong outflow occurs in the anvil at 12 km AGL . These are features similar to those seen in the single radar data fields (Fig. 1a, b). Although the cross-section in Fig. 2b shows mostly updrafts, other regions of the storm have downdrafts. The chilled downdrafts are sometimes so

Table 1 Comparison of tornado warning performance, 1977–78

	Present system	Doppler radar alone
Probability of detection*	0.64	0.69
False alarm rate†	0.63	0.25
Critical success index‡	0.30	0.56
Lead time (min)§	2.2	21.4

Approximately 200 cases were examined.

* $X/(X+Z)$; † $Y/(X+Y)$; ‡ $X/(X+Y+Z)$ where X = number of predicted tornadoes that occurred; Y = number of predicted tornadoes that did not occur; Z = number of tornadoes that occurred without a prediction.

§ Lead time between issuing of warning and tornado occurrence.

strong and enduring that they form cooled air masses that extend many kilometres beyond the storm cloud.

Observed winds compared with model predictions

High-speed, high-capacity computers allow us to model numerically thunderstorm evolution. As computers become faster and less expensive and models more computationally efficient, it even becomes feasible to use prestorm environmental parameters (vertical profiles of temperature, moisture and wind) for improved predictions of weather events. Figure 3 shows a comparison of a numerically modelled storm formed in an environment similar to that of an observed storm³² in which wind at 4 km AGL is from the SSW (199°) at 25 m s^{-1} . Winds are relative to storm motion (14 m s^{-1} at 192° for the model; 18 m s^{-1} at 193° for the observed storm). The heavy contour in Fig. 3a corresponds to a reflectivity factor of 38 dBZ, assuming the Marshall–Palmer dropsize distribution. Seen in both the modelled and observed storms are the updrafts and downdrafts with reasonable agreement in shape, location and intensity. The updraft blocks the dry environmental air at 4 km and diverts it around the storm whose circulation causes higher speed dry air to intrude into the storm's east side. There the fast-moving dry air deflects and evaporates the condensate, thus forming a notch-like feature in the reflectivity contour, seen in both the model and observations.

Tornado observations

When a tornado is contained within the radar's resolution volume, the Doppler velocity spectrum becomes very broad³³. When radar targets have small terminal velocities, the Doppler spectra can be analysed to determine the flow of air around the tornado^{34,35}. The only scanning of a large tornado (Fig. 4a) with a pulsed Doppler radar operating with a Nyquist interval sufficiently large ($\pm 91 \text{ m s}^{-1}$) to measure unambiguously the high tornadic wind speeds was in Spring 1981 at NSSL. The Nyquist interval, $\pm \lambda/4T_s$, is determined by radar wavelength λ and the period T_s between transmitted pulses. Targets moving at speeds higher than the Nyquist limits produce signals that are not sampled often enough to resolve unambiguously the Doppler shift. The contour in Fig. 4a shows the approximate location and cross-section of 16 consecutive (in range) measurement volumes within which targets produced the 16 Doppler spectra shown in Fig. 4b. Each spectrum gives the distribution of velocities within one of the 16 measurement volumes, and each volume has a range extent of $\sim 200 \text{ m}$. These spectra were recorded within a minute of the time of the tornado photograph in Fig. 4a. Because the antenna was scanning in azimuth while data were collected to form these spectra, the measurement volume has an azimuthal dimension of 1.5° , although the beam-width is 0.8° (ref. 25). The 16 volumes are aligned along the

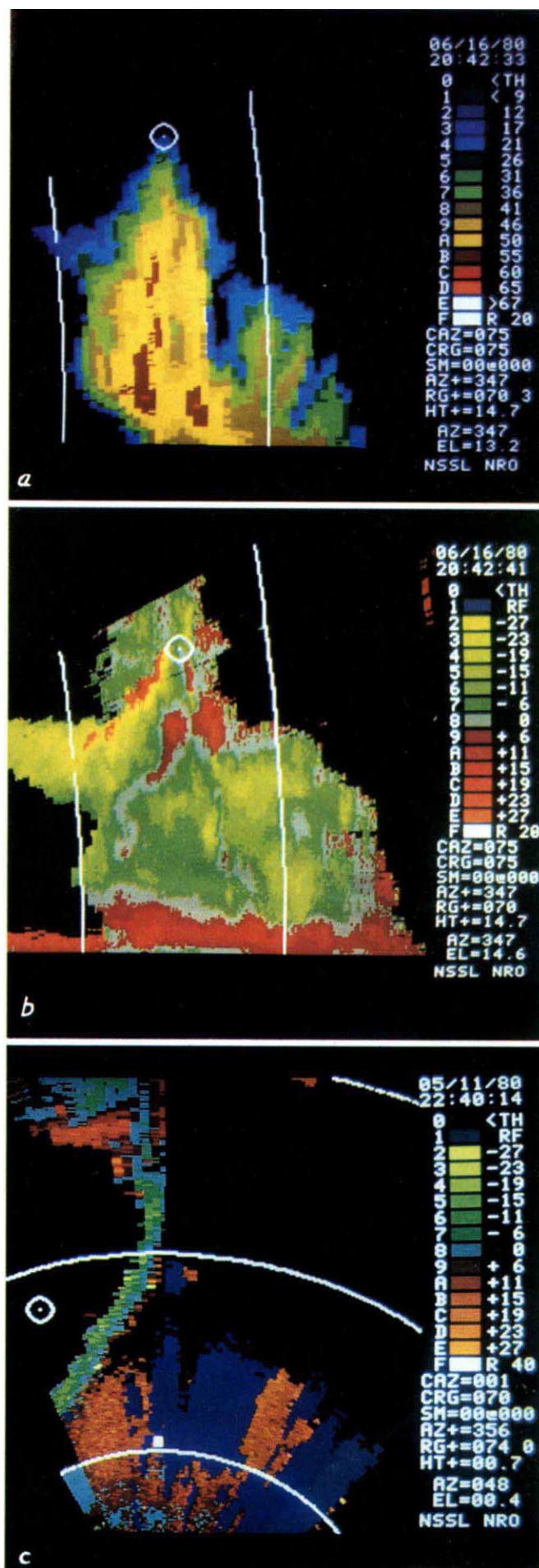


Fig. 1 *a*, Vertical cross-section of reflectivity factor Z , a measure of precipitation intensity, through a severe storm that occurred in central Oklahoma on 16 June 1980. The storm top (indicated by dot in white circle) is at 14.7 km AGL. The two shades of blue indicate median values of $10 \log_{10} Z$ (dBZ) equal to 17 and 21; the three shades of green correspond to 26, 31 and 36 dBZ; the three of yellow to 41, 46 and 50 dBZ; and the two of red to 55 and 60 dBZ. The relation between dBZ values and rainfall rate is given in the text. The radar location is to the left, and the vertical arcs are at 60 and 80 km from the radar. *b*, Radial velocity field in the same vertical section as *a*. Red (green) coloured areas are radial velocities away from (towards) the radar. The shades from dark to light correspond to median radial velocities of 6, 11, 15, 19, 23 and 27 m s^{-1} . The grey hue is for a median value of 0 m s^{-1} . Differences in storm outline from that in *a* are discussed in the text. *c*, Radar depiction of radial velocities in a bow gust line produced by thunderstorm outflow at ground level. Colour code is the same as in *b*. This is an azimuthal sweep through the gust at a constant elevation angle of 0.4° , and the arcs are at 40 and 80 km range.

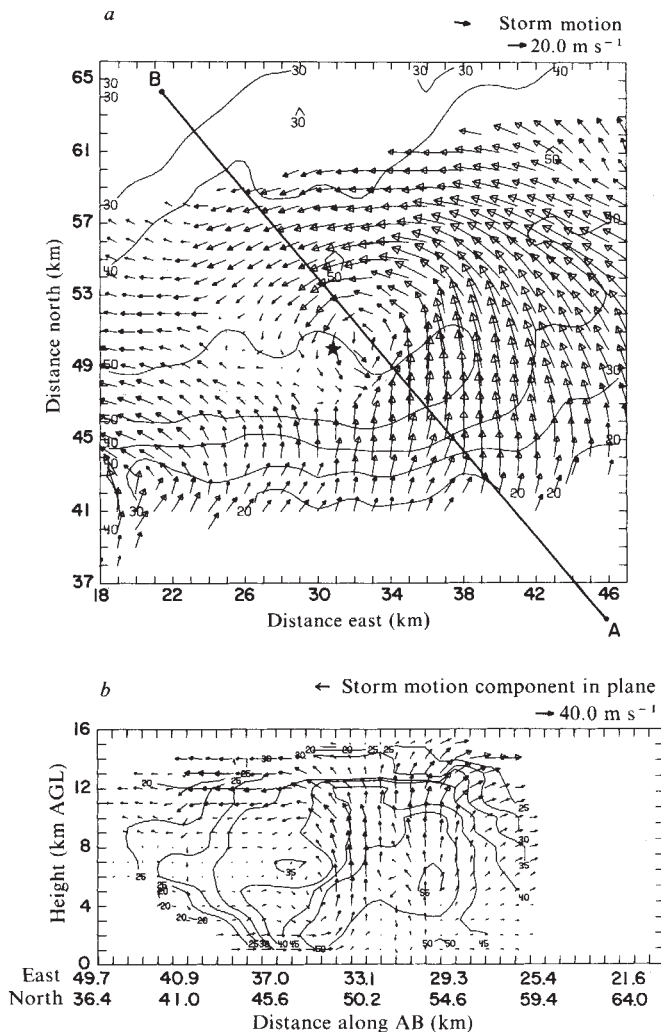


Fig. 2 Contours of reflectivity factor in dBZ and the wind field in a horizontal cross-section at 2 km AGL (a) and a vertical cross-section (b) of a tornadic thunderstorm. Winds are in the coordinate system moving with the storm. Storm motion speed and direction are shown. Line AB in a is the location of the vertical cross-section. Distances are from a radar at Roman Nose State Park, Oklahoma²⁹. ★, The tornado location in a. The arrow of indicated velocity at top right of each plot gives speed which is proportional to arrow length (E. Brandes and B. Johnson, personal communication).

beam, but only two or three of these cross the tornado's circle of maximum wind. In Fig. 4b the widest spectra denote the location of the tornado. Maximum tornado wind speeds can be estimated from these data. At this time the radar-measured maximum winds were $\sim 90 \text{ m s}^{-1}$. Acquiring *in situ* data in and around the tornado is very difficult, but Doppler radar provides a remote and relatively safe method of surveying storms for tornadoes.

Although in principle two or more Doppler radars are required to depict circulations inside storms, a single Doppler radar can indicate circulations, such as mesocyclones, that have nearly circular symmetry. The circulation signature of large mesocyclones shows itself remarkably well on colour displays (see cover of ref. 36). Because mesocyclones are often several kilometres in diameter (see Fig. 2a), their circulations can be resolved to ranges of $\sim 200 \text{ km}$ with radars having beam widths $< 1^\circ$.

A test was conducted in Oklahoma to determine how tornado warnings are improved over the present non-Doppler warning system when Doppler information alone is used to forecast tornadoes. Table 1 compares several measures of effectiveness

of tornado warning using Doppler radar alone with present techniques which rely heavily on visual sightings. The critical success index (CSI)³⁷ gives credit for a high probability of detection and low false alarm rate, with a CSI of 1.0 being perfect.

Doppler radar can increase the average lead time before tornado occurrence by 20 min, thus offering the population a longer time to take cover if warnings are communicated properly. Although the probability of detection at the instant of issuance of warning is not significantly improved, the false alarm rate is significantly decreased with Doppler radar. Therefore, Doppler radar can reduce the overwarning inherent in the present system even while giving longer lead times, and warning areas may be reduced substantially by precise location of the storms containing mesocyclones.

Gust fronts

A gust front is the leading edge of cool air produced when a raincooled downdraft turns into an outward moving, ground-based flow. The front is marked by shifts (shear) in the wind, both in the vertical and horizontal directions. A gust front can propagate in the clear air many tens of kilometres away from the thunderstorm that caused it and yet harbour shear forces that can be destructive to aircraft, especially when a flight crew is unaware of its presence. The wind behind the front is usually turbulent and attached to the storm's downdraft³⁸. But in some cases, as shown in Fig. 1c, the outflow air can detach itself from the storm and propagate away from it. Gust fronts often appear as thin lines on radar displays Fig. 1c, and reflectivity is so weak ($< 10 \text{ dBZ}$) that some radars fail to detect it. However, moderately sensitive Doppler weather radars can sense reflectivities as low as -10 dBZ at ranges $< 60 \text{ km}$.

Figure 1c shows the Doppler velocity field of a gust produced by a thunderstorm, which can be partially seen at the top of the figure. The radar beam elevation was fixed at 0.4° while the radar scanned in azimuth from 330° to 45° . Note the abrupt change in radial velocity across the gust line. The wind on the radar side (south-east) of the line shows a component away from the radar, and just beyond the line, towards the radar. The region of approaching velocities along the gust is rather narrow. The air flow immediately behind the 4-km wide band of approaching air is again southerly, although it is not evident in Fig. 1c because the SNR threshold eliminated the weaker echoes behind the gust. The dark blue area near the 40 km range arc indicates regions where the velocity data are contaminated with echoes from distant ($> 150 \text{ km}$) storms along the same bearing. A time sequence of radar images of the thunderstorm and gust suggests that this outflow propagated more than 80 km with a relatively constant velocity of 13 m s^{-1} . Fortunately, the gust passed the 444-m tall KTVY television tower, instrumented by NSSL, so that *in situ* measurements of the wind and temperature were obtained.

The vertical profiles of the wind and temperature in a south-east to north-west cross-section obtained from a composite of radar and tower data are shown in Fig. 5. The data show this solitary gust to be a cylindrical pool of cool air that propagates southeastward. This highly localized pool of steadily moving cool air appears to be detached from the storm and propagates in a stable boundary layer with little mixing of its air with the environment so that the air behind the line quickly returns to ambient conditions. This solitary gust seems to have a remarkably long lifetime ($> 1 \text{ h}$) as judged from time lapse photography.

Turbulence

Evident in Figs 2 and 5 (and in the visual images of cumulus clouds) are small scale eddies, perturbations in the larger scale circulations of storm winds. Radars with finer resolution than the nearly 2 km resolution of Fig. 2 and *in situ* point measurements made with aircraft often show small scale thunderstorm

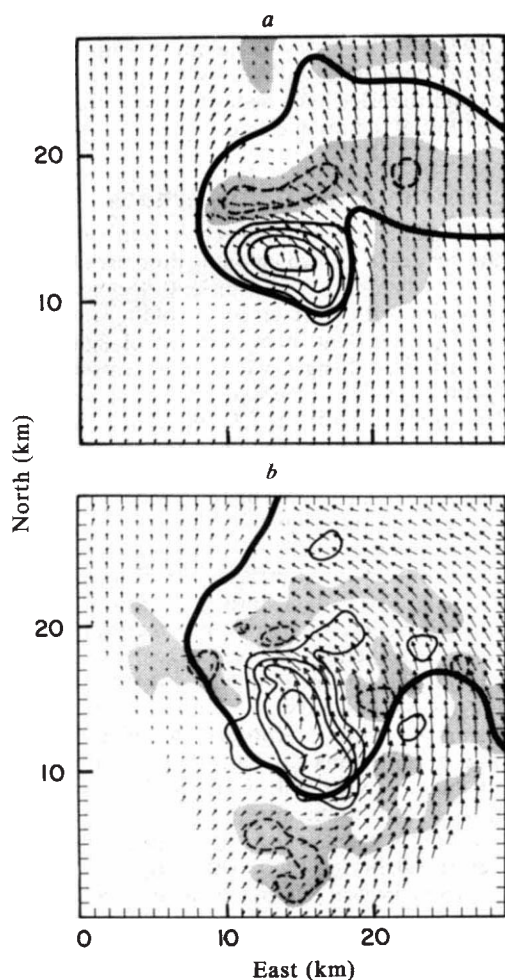


Fig. 3 Horizontal cross-section of modelled storm winds (*a*) and winds synthesized from observed Doppler velocities (*b*) in a plane at 4 km AGL (ref. 32). Updraft velocities (solid lines) and downdraft velocities (dashed lines) are contoured in 5 m s^{-1} increments. Shaded areas are downdraft regions stronger than -1 m s^{-1} . The heavy solid contour in *a* encloses a region of liquid water concentration $>0.5 \text{ g kg}^{-1}$. In *b* the heavy contour is a 30 dBZ reflectivity factor. Wind vectors are scaled such that one grid interval is 20 m s^{-1} .

wind to be chaotic. If these turbulent eddies are intense, they can be destructive to aircraft.

The width, σ_v , of the Doppler velocity spectrum is normally a measure of turbulence within the measurement volume, although shear, antenna scan rate, and differential fall speeds contribute to a lesser degree to the measured value^{24,39}. Although an aircraft can record turbulence in narrow volumes along its flight path, and a radar resolves it within its usually much larger measurement volume, Böhne⁴⁰ has shown good correlation between levels of turbulence estimated from aircraft and radar. Analysis of a larger amount of data by Lee⁴¹ suggests a strong correlation between σ_v and *in situ* aircraft measurements of turbulence. His data show that when aircraft-measured gust velocities exceed 6 m s^{-1} (moderate or severe turbulence), σ_v exceeds 5 m s^{-1} within 1 km of the radar measurement volume. However, not all storm regions of large σ_v produced turbulence detected by aircraft. Furthermore, in over 50 thunderstorm flights when σ_v was $<4 \text{ m s}^{-1}$, the aircraft experienced only light turbulence.

Only meagre results relate radar-estimated turbulence to storm wind fields; nevertheless, present evidence suggests that the most intense turbulence in a tornadic thunderstorm occurs

along the edges of high speed ($\geq 20 \text{ m s}^{-1}$) updrafts⁴². The most intense turbulence is usually found in the midlevels of the storm where there is large shear between updrafts and downdrafts⁴³.

Rainfall measurements

Although radar estimates of rainfall rate R obtained from Z are typically in error by a factor of up to two, radar has the advantage of surveying remotely vast areas and making millions of measurements in minutes. A sufficiently dense network of rain gauges can measure the rainfall more accurately and reliably. However, no matter how accurate the gauges are for point measurements (typically 5–10%), their usefulness for determining the rainfall integrated over a large area is limited by gauge density and the spatial variability in the rainfall⁴⁴. The cost of a rain gauge network to match the radar's capabilities

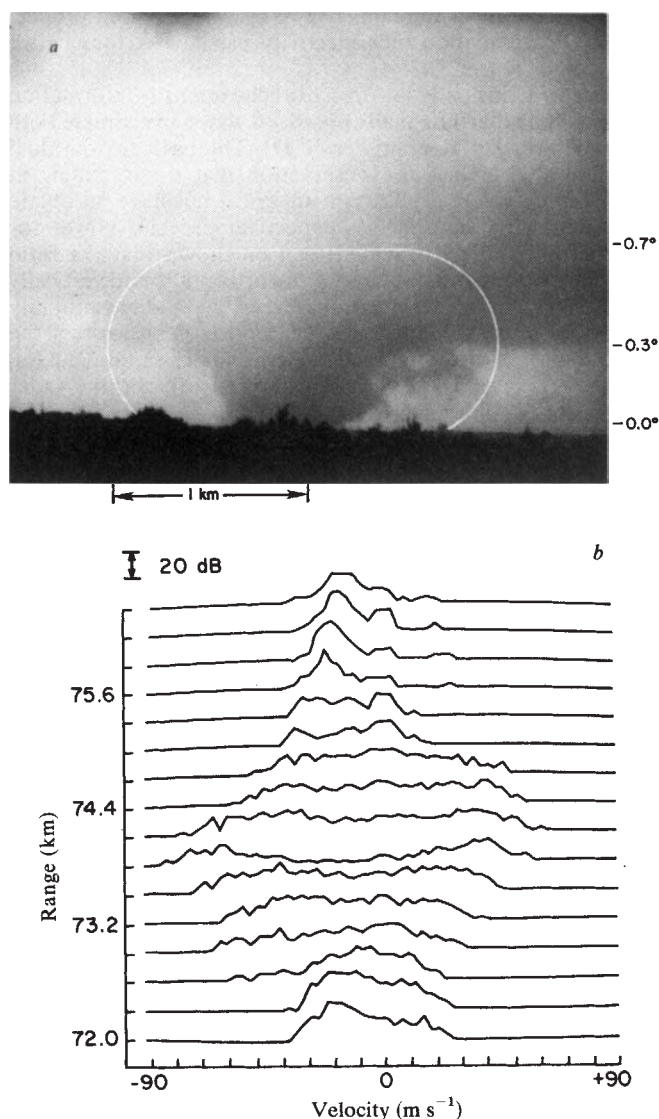


Fig. 4 *a*, The tornado near Binger, Oklahoma, at ~16.06 CST on 22 May 1981. View is to the NW and range from the photographer is 5.2 km (R. Davies-Jones and D. Burgess, personal communication). The white contour is the approximate size of the radar measurement volume for the data shown in *b*. *b*, Reflectivity spectral densities (in dB units) versus Doppler (radial) velocity at 16 range locations in the tornado's circulation. Beam elevation angle is 0.3° . The nearly horizontal lines at each end of the spectra are thresholds at 10 dB above the receiver noise level and correspond to a reflectivity spectral density of $\sim 3.4 \text{ mm}^6 \text{ m}^{-3} \text{ s}^{-1}$. The vertical scale is 20 dB per division (L. Hennington and D. Zrnic, personal communication).

in spatial continuity and rates of data sent to a central location would be prohibitive. Therefore, radar and widely-spaced rain gauge data are combined to produce rainfall maps. Inherent in this procedure are the assumptions that, despite the possible error in absolute radar measurement of R , the radar accurately measures the spatial variability of R and a valid calibration can be made between gauge and radar measurements at each gauge location. With these assumptions such rainfall maps approach the point accuracy of a dense gauge network^{45,46}.

The distribution of dropsize, $N(D)$, is of central importance in determining R . Operational weather radars measure the single parameter Z , and meteorologists often estimate R by using an assumed exponential dropsize distribution (see ref. 28) in which only one parameter is estimated from Z . In general, however, specification of $N(D)$ requires knowledge of several independent parameters to describe it, so that accurate estimates of R and Z can be made. It has been suggested that when $N(D)$ is described by a two-parameter distribution, pairs of independent measurements of two remotely sensed properties, such as attenuation and reflectivity, can be used for a better estimation of R (ref. 47).

A dual polarization radar⁴⁸ can use echo intensity information contained in two orthogonally polarized waves to estimate both parameters of a two-parameter $N(D)$. The basis for the dual polarization scheme is the observation that drops, falling at their terminal velocities, are not spherical but have an oblate spheroidal shape, so that we expect larger echo power for horizontally polarized waves. It can be shown that the ratio Z_H/Z_V of reflectivities obtained with horizontally and vertically polarized waves is an accurate measure of the spatial variability of R , even if the absolute radar calibration is in error, if the assumption of exponential dropsize is valid. Then rain gauges, even though sparsely spaced, can be used to scale the radar estimates of R . Although results given by Seliga *et al.*⁴⁹ suggest that a dual polarization radar can give better estimates of rainfall rate, more research is needed to evaluate the technique for operational applications.

Because the ratio Z_H/Z_V is related to the shape of the scatterers, polarization analysis can also be used to identify precipitation type (rain, hail, graupel, and so on) when this ratio and absolute measurements of Z are categorized into levels expected for various types of hydrometers⁵.

Lightning studies using radar

Lightning flashes occur as either cloud-to-ground (CG) or intra-cloud (IC). A CG flash is comprised of one or more return strokes, which have large electrical currents in the channel between the cloud and ground, while all flashes not reaching the ground are termed IC. (See ref. 50 for a general description of lightning and its measurement.) Both IC and CG flashes create ionized channels that carry electrical currents. Backscattering from these ionized channels is sufficient to produce radar echoes.

Radar detection of lightning has been known for many years^{51–58}. Although some early studies included estimates of

values for lightning parameters such as electron density, it was not until 1972 that a theory of lightning echoes from return stroke channels was proposed⁵⁹. Since then, new measurement techniques and theories have been developed to help unravel the complexities of lightning echoes.

Characteristics of lightning echoes

In a few early studies of lightning echoes, attempts were made to relate the echoes to known lightning processes by comparing the sferics from lightning (the transient electromagnetic field radiated by a flash) with the radar echoes of the same flashes^{57,60}. More recently, coincident measurements of lightning echoes and electrostatic field changes, which allow reasonably conclusive identification of flash type and inter-flash events, showed that lightning echoes from a CG flash often have sudden increases in amplitude associated with return strokes followed by a decay to pre-flash levels⁶¹ (Fig. 6). This feature has also been noted during some interstroke intervals. The radar echoes from an IC flash usually have a relatively broad peak with variations superimposed, which may be seen in Fig. 6b during the first part (0.6–1.15 s) of the flash. The electric field changes, (R in Fig. 6) are typical of return strokes. This flash was independently verified by a lightning strike location system as a CG with at least eight return strokes. Note that abrupt increases in the lightning echoes in a particular range interval ΔR may not always coincide with the return stroke field change.

Comparison of lightning echoes with electric field changes suggests that a continuing discharge process often keeps the channels ionized^{62,63}. We define the duration of lightning echoes as the time interval from the instant the echoes are first discernible until they decay back to the pre-discharge amplitude. This duration forms a lightning echo event. Figure 6 shows at least nine echo events. Typical lightning echo events have durations of ~200–300 ms with extreme values of 10 ms and 3 s having been observed.

The rise time (the time for the radar received signal to reach peak amplitude) of a lightning echo event is generally a few tens of milliseconds. Although it was first thought that the rise time indicates increasing current flow in the channel⁵⁷, more recent analysis⁶⁴ shows that observed rise times agree well with ones determined theoretically, when the lightning is modelled as an ionized channel or streamer propagating across the antenna beam at a rate of ~100 km s⁻¹. Propagation speeds of this order of magnitude for streamers within clouds have been inferred by several investigators, using different measurement techniques^{65,66}. When streamers propagate radially along the beam, their echoes can be seen developing in contiguous resolution volumes. Radar observations of several thousand lightning flashes in Oklahoma storms have yielded a maximum propagation speed of ~250 km s⁻¹, and streamer propagation speeds of >100 km s⁻¹ have been measured within small storms⁶².

The radar cross-section, RCS, of lightning has been estimated both theoretically and experimentally. The lightning RCS depends on two factors: (1) the size and number of reflecting

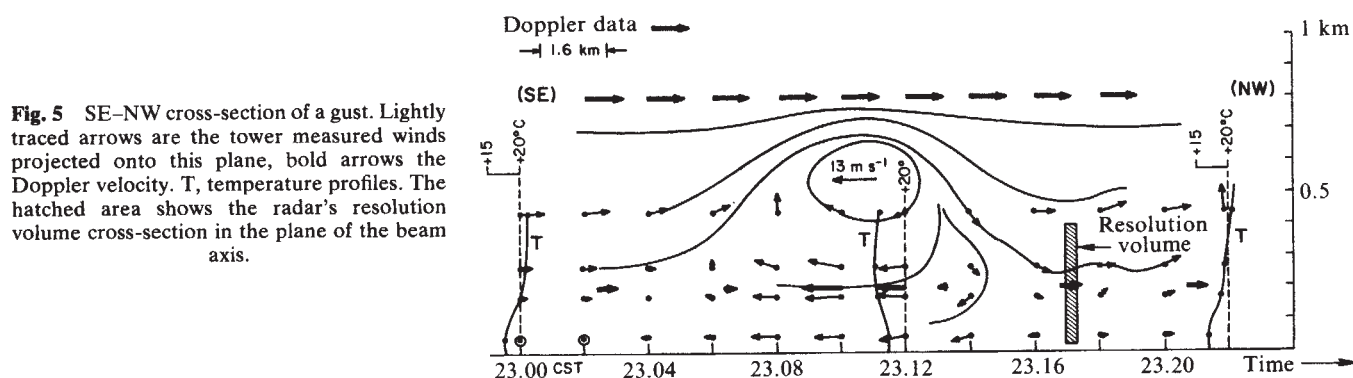


Fig. 5 SE-NW cross-section of a gust. Lightly traced arrows are the tower measured winds projected onto this plane, bold arrows the Doppler velocity. T, temperature profiles. The hatched area shows the radar's resolution volume cross-section in the plane of the beam axis.

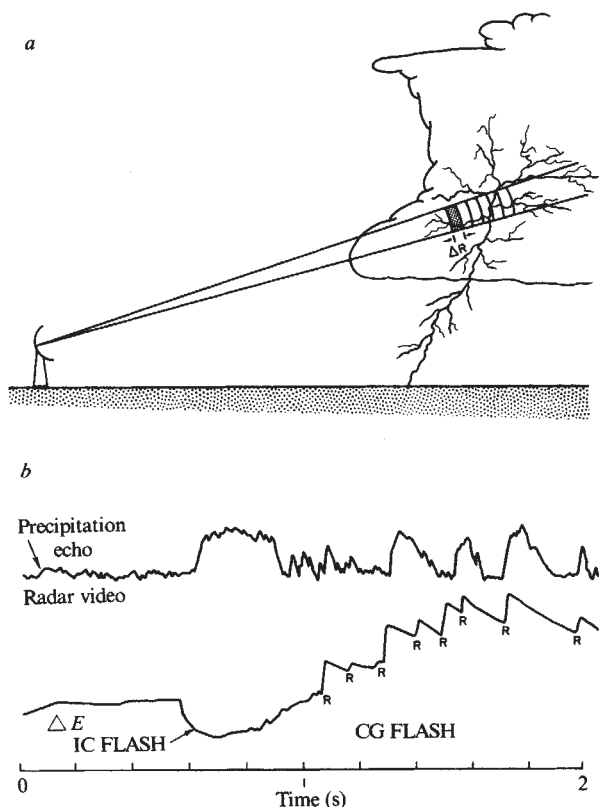


Fig. 6 Depiction of a technique (a) for observing lightning and an example (b) of lightning echoes for a long flash that began as an IC and then became a CG. The radar antenna typically is held at a fixed position to acquire lightning echoes. The stippled region in a, ΔR , represents one of the radar's resolution volumes in which the time variation of echo intensity may be studied. Top trace of b is time history of lightning echo intensity in a resolution volume at 86 km and 4 km altitude. Although depicted as a continuous line, the trace is actually a series of points separated in time by the period T_s (≈ 1 ms) between transmitted pulses. Precipitation echo denotes pre-flash radar echo intensities due to precipitation. The bottom trace is the electrostatic field change at the radar site due to the same flash. (Reprinted from ref. 21.)

lightning channel elements within the radar resolution volume and their orientations relative to the incident wave, and (2) the channel plasma characteristics. The initial theoretical work was principally done by Dawson⁵⁹, who assumed that only those ionized channels perpendicular to the beam axis contributed significantly to lightning echoes, and Divinsky⁶⁷, who considered the return echoes irrespective of channel orientation. Both treated the limiting cases of low density and overdense plasma (the plasma is considered overdense when the radar signals are reflected from it as if it were a metallic conductor). More recently Mazur⁶⁴ estimated the RCS as a function of channel orientation relative to the incident wave and any level of electron density and temperature by treating lightning elements as dielectric cylinders of finite length. Experimentally determined estimates of the lightning RCS reported by Mazur⁶⁴, Holmes *et al.*⁶², and Zrnice' *et al.*⁶³ are reasonably consistent. Holmes *et al.*⁶² conclude that the in-cloud portion of their flashes, which were at altitudes above 6 km, had channels with diameters < 2 cm and electron densities of $\sim 10^{16} \text{ m}^{-3}$.

Lightning and storm structure

The fact that radars of wavelength ≥ 10 cm can easily locate lightning, even in intense precipitation, out to ~ 300 km makes them useful in the study of lightning relative to storm structure. The use of Doppler radar to observe lightning is a promising new area of research.

A phenomenon of long-standing interest and controversy is the 'rain gush', which is a dramatic increase in rainfall soon after lightning. The controversy has arisen as a result of different interpretations of the gush: one is that lightning causes the rapid growth of precipitation⁶⁸, another is that the precipitation, in electrostatic levitation before the flash^{69,70}, is merely released by the change in the electric field caused by the flash. Other interpretations have also been suggested.

Recently two radars have been used by Szymanski *et al.*⁷¹ to search for a relationship between rain gushes and lightning. A 3-cm wavelength radar was used to observe precipitation reflectivity while an 11-cm wavelength radar was used to record the location of lightning echoes. They report that of more than 150 flashes observed by radar, only one flash, which occurred in very low precipitation reflectivities, seemed to cause precipitation growth in the volume around the lightning. Further such observations with radar are needed to provide more definitive information concerning the fundamental question of electrical effects on precipitation.

Zrnice' *et al.*⁶³ found, as had Szymanski *et al.*⁷¹, that lightning in heavy precipitation usually causes no discernible change in precipitation reflectivity. However, Zrnice' *et al.* did find that lightning altered precipitation reflectivity of low Z and suggested that these were due to changes in orientation brought about by electrostatic field changes in these regions. These changes occurred within 0.1–0.5 s after the flash began. McCormick and Hendry³ give convincing evidence that both aerodynamic forces and the storm's electric field control the orientation of ice crystals.

We have combined measurements of precipitation, using our 10-cm wavelength Doppler radar, and lightning echoes, using our 23-cm wavelength radar, to study the coevolving lightning and precipitation structure of large storms. In a squall line (that is, storm cells along a line) on 21 June 1980, over 1,000 lightning flashes were observed during a 44-min period. The storm cells were aligned nearly radially from the co-located radars, and lightning flashes from several cells were recorded. For this one squall line, the following were noted⁶⁴: although lightning is observed throughout the storm, most of the lightning occurs close to the leading edge (relative to storm movement) of the heavy precipitation; the incidence of long lightning flashes that propagate between growing and dissipating cells increases as the precipitation echo intensity decreases in the dissipating cell.

The length of lightning flashes determined from the radial extent of lightning echoes varies greatly. Holmes *et al.*⁶² report lengths up to 2 km in small, isolated mountain thunderstorms. Ligda's⁵² photographs show lightning with lengths up to ~ 160 km in large storms. Most lightning that we observe in Oklahoma is several tens of kilometres long, and we have

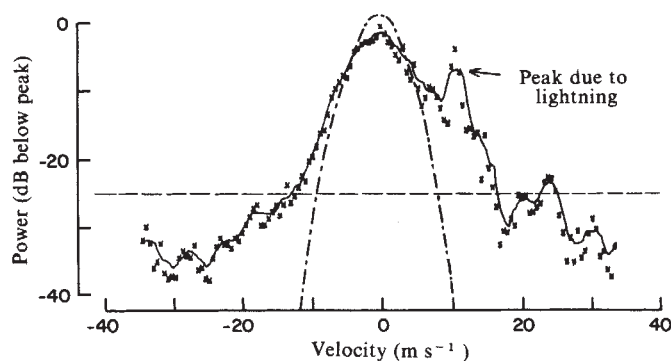


Fig. 7 Reflectivity spectral density for vertical fall velocities from precipitation and lightning at 7.1 km. The dash-dotted curve is the estimated true fall speed spectrum. The solid curve is a five-point running average of the data ($\times$), which were obtained by averaging 12 spectra. Powers below the dashed line and the peak at 24 m s^{-1} are considered to be unreliable, so the intersection of this line and the observed spectrum yields the assumed, reliable maximum fall velocity of $\sim 14 \text{ m s}^{-1}$.

observed lengths in excess of 100 km. These very long flashes have occurred in severe storms or along a line of storms.

Lightning often appears visually to occur in closely-spaced sequences, suggesting that the first flash somehow causes others. Mazur⁶⁴ has studied this phenomenon with radar. He observed lightning events characterized by a time interval of <200 ms between them and with a separation in the range of only a few kilometres. He has recorded these in squall lines both in the East Georgia region of the USSR and in the Great Plains of the USA. Mazur termed these 'associated discharges' because of the apparent association between the seemingly separate lightning flashes. It is, of course, possible that different branches of the same flash propagated by chance into the beam to give the observed apparent association. However, Mazur's analysis of lightning in an intense storm showed that this possibility was <5%. From this, he concluded that there may be processes that result in one flash being initiated by another.

Zrnice' *et al.*⁶³ have used a vertically pointing, 10-cm wavelength Doppler radar to study interactions between lightning and precipitation. Figure 7 shows an example of the reflectivity spectral density of lightning and precipitation observed simultaneously by Doppler radar. Reflectivity spectra obtained with vertically pointed radars have been used to determine the drop size distributions^{72,73}. Unless the vertical air velocity and turbulence are estimated with precision better than 1 m s^{-1} , large errors in drop size distribution will result. Although several methods have been proposed, none will provide correct values of vertical air motion⁷⁴. If lightning produces ionized channels that permeate the measurement volume, these channels should be perfect tracers of the updraft and turbulence because they have zero terminal velocity.

A subjectively estimated true reflectivity spectrum for the hydrometers is also plotted on Fig. 7 to show how the spectrum depends on velocity. The true spectrum has an upper speed

limit equal to the mean fall speed of the smallest detectable drops. Departures of the observed spectrum from the true is caused by turbulence, window broadening (a signal processing effect), possibly returns received via sidelobes, or other non-meteorological effects. Assuming that the ionized channels drift with the wind, Zrnice' *et al.*⁶³ estimated the updraft velocity to be 10 m s^{-1} , and so the maximum reliable terminal velocity estimate for the largest hydrometer is $\sim 24 \text{ m s}^{-1}$. Such a high velocity was attributed to hail, which indeed fell in the vicinity of the radar. Although this newly tested Doppler radar technique shows potential to determine true updraft velocity and to estimate the radar volume-averaged drop size distribution, much more research is required to ascertain the accuracy and utility of this method.

Concluding remarks

The usefulness of weather radar as a remote probe of large and often severe thunderstorms continues to expand. When used in combination with other sensors, it offers us the opportunity to learn more about the complex interrelations between wind, water, and electricity in storms. Much of this understanding can then, in turn, be applied to the many weather-related problems that affect numerous aspects of our daily lives.

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ARTICLES

Tectonic subsidence, flexure and global changes of sea level

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Tectonic models for the evolution of passive continental margins predict that following rifting, sediments should progressively onlap basement at the edge of a margin as the lithosphere cools and increases its flexural rigidity with age. The pattern of modelled onlap is strikingly similar to that used by Vail and colleagues to estimate sea-level rise through geological time. This similarity suggests that major portions of stratigraphical sequences at margins may have a tectonic, rather than eustatic, control. The patterns of onlap used by Vail and colleagues may be widespread, however, because several widely separated passive margins rifted at similar times, but they are unlikely to be worldwide.

THE distribution of seas through geological time is the result of two principal factors: global or eustatic changes of sea level and tectonic movements of the Earth's crust¹. One or both of these factors may cause shorelines to shift and sedimentary facies to change. It may be expected that a relative rise in sea level, due either to a global rise in sea level or to crustal subsidence, would produce a transgressive stratigraphical sequence, while a relative fall of sea level, due either to a global fall in sea level or to crustal uplift, would produce a regressive sequence. The occurrence of a transgressive or regressive sequence at a particular locality in a sedimentary basin, however, is controlled by the rate of sediment influx and the rate and direction of change in the relative position of sea level and the sea floor². It appears that global changes in sea level, due to changes in the volume of land ice or mid-ocean ridge crests, proceeded rapidly enough to explain some of the transgressive and regressive sequences at the edges of epeiric seas during the geological past. But there has been considerable debate³ on the origin of the transgressive and regressive sequences that occurred during periods of no extensive land ice and relatively constant ridge crest volumes.

Sloss and Speed⁴ argued that the major control on stratigraphical sequences in the continental interiors⁵ were tectonic movements. They recognized three main episodes or 'modes' during the development of stratigraphical sequences in the continental interiors: 'oscillatory', in which land areas were subject to differential uplift and subsidence; 'emergent', in which land areas were progressively uplifted; and 'submergent', in which land areas progressively subsided. Sloss and Speed⁴ noted a similar timing of these modes between widely spaced continents and argued for a synchronous tectonic control over broad regions.

Vail *et al.*^{6,7}, on the other hand, argued that the major control of sedimentary sequences in continental interiors and margins was global changes of sea level. They recognized several depositional cycles during the development of a stratigraphical sequence, each of which was bounded by surfaces of discontinuity. By identifying these surfaces, using characteristic patterns of seismic reflectors of 'onlap' and 'offlap', they constructed cycle charts at individual localities. Vail *et al.*⁶ noted that similar cycles could be recognized at localities in widely separated margins and argued for a eustatic control. They used estimates of the amounts of onlap and offlap to construct a global sea-level curve for the Phanerozoic that was characterized by several short-term fluctuations superimposed on a broad long-term change. Vail *et al.*⁷ used the pattern of onlap and offlap to infer sea-level rise and fall respectively. By calibrating their 'global' curve with other estimates of long-term sea-level changes^{8,9} they argued that the short-term fluctuations could have magnitudes of up to a few hundred metres¹⁰.

Pitman⁹ pointed out that the occurrence of a transgressive or regressive sequence at the edge of a gently sloping shore depends on the rates of change of tectonic movements and global sea level. For example, a transgressive sequence could result from a sea-level fall, if the rate of subsidence of a basin exceeds the rate of sea-level fall. Pitman⁹ showed that a major transgression during the Eocene in the North American and African margins could result simply from the interaction of changes in the rates of long-term sea-level fall with the steady, normal subsidence of these margins.

Vail and Todd¹¹ therefore modified the original statements⁷ that coastal onlap and offlap could be directly equated to sea-level rise and fall. Their amended global sea-level curve for the Jurassic, however, was strikingly similar to the original curve, the main difference being the less abrupt sea-level falls.

The nature of the control of stratigraphical sequences has subsequently been widely debated. Hallam¹² argued that a tectonic control on the scale proposed by Sloss and Speed⁴ was unlikely, as there was no satisfactory mechanism to explain why widely separated continents would be affected by tectonic movements at similar times. He suggested¹³ that the main control on stratigraphical sequences were global changes of sea level and constructed a curve for the Jurassic, similar in overall shape to the Vail *et al.*⁶ curve. But as Donovan and Jones³ have pointed out, without a satisfactory mechanism, the

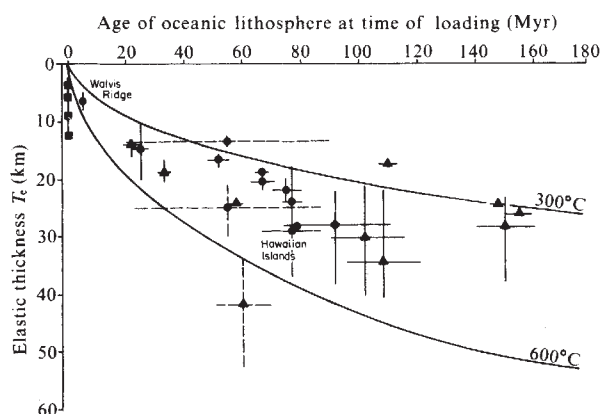


Fig. 1 Plot of elastic thickness T_e against age of the oceanic lithosphere at the time it was loaded⁴⁸. The elastic thickness is determined from observations of the flexural rigidity and assumes Young's modulus = 10^{12} dyn cm⁻² and Poisson's ratio = 0.25. ●, Seamounts and oceanic islands; ▲, deep-sea trench-outer rise systems; ■, ridge crests; ◆, river delta. Solid lines indicate the 300 °C and 600 °C oceanic isotherms based on the cooling plate model⁴⁹.

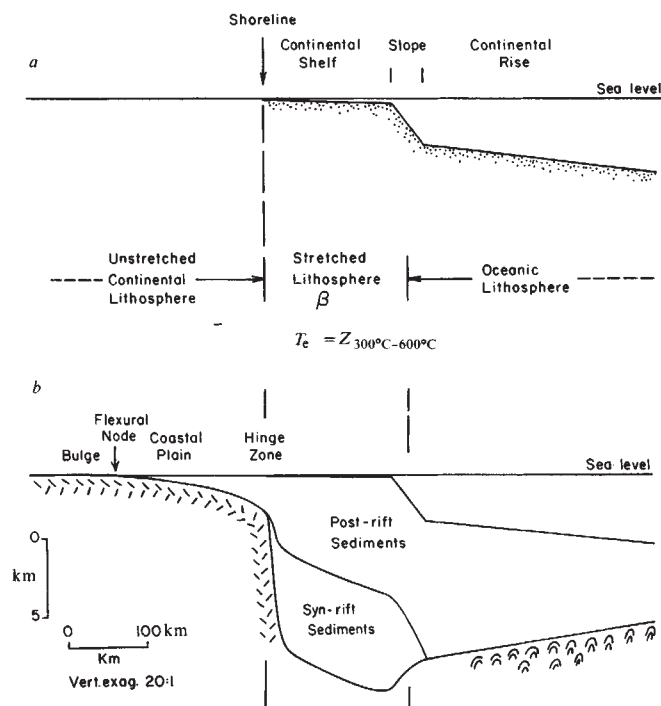


Fig. 2 Thermal and mechanical model for the tectonic evolution of a passive continental margin. *a*, Initial conditions. The tectonic subsidence of the margin is due to thermal contraction following heating and thinning of the lithosphere and crust at the time of rifting. Sediments are assumed rapidly to infill continental shelf, slope and rise regions and to maintain a constant bathymetric profile through time. *b*, Cooling and flexure. The sediments represent a load on the cooling lithosphere which responds by flexure. Sediments that form early in margin history load a relatively weak lithosphere while sediments that form later in its history load a strong lithosphere. The sediments that infill the initial subsidence are referred to as syn-rift while the sediments that infill the thermal subsidence are referred to as post-rift³¹. The model calculations assume an initial lithospheric thickness of 125 km, an initial crustal thickness of 31.2 km, a coefficient of volume expansion of $3.4 \times 10^{-5} \text{ } ^\circ\text{C}^{-1}$, a mantle temperature of $1,333^\circ\text{C}$ and initial densities of 2.8 and 3.33 g cm^{-3} for the crust and lithosphere respectively. A uniform density of 2.5 g cm^{-3} is assumed for the sediment infill.

large amplitude sea-level changes inferred by Vail *et al.*⁶ and Hallam¹² must also remain in doubt. Bally¹⁴ suggested, in fact, that the effects of global sea-level changes are subordinate to tectonics and that the major control on stratigraphical sequences are widespread and correlatable tectonic events associated with major plate reorganizations.

There is little doubt, however, that the sea-level curve of Vail *et al.*⁶ represents an important synthesis of high-quality stratigraphical data that apparently has been useful in correlation studies and petroleum exploration. The outstanding question is the interpretation of these curves and whether they represent global sea-level changes or widespread tectonic events. Unfortunately, many of the data used by Vail *et al.*⁶ are not generally available so it is difficult to determine whether they have satisfactorily separated eustatic from tectonic effects. This study uses recently developed models for the tectonic evolution of passive margins^{15,16} to estimate how tectonics has contributed to stratigraphical sequences.

Tectonic subsidence at passive margins

The knowledge of the tectonic evolution of passive margins has progressed rapidly during the past decade. Backstripping studies¹⁷⁻²¹, using data from commercial exploratory and COST-type wells, have shown that the principal factors affecting the post-rift subsidence of passive margins are thermal contraction and sedimentary loading. Factors such as chemical and

mechanical compaction, palaeobathymetry, amount of sediment influx and long-term changes in sea level all contribute to the subsidence but their combined effects are small compared with thermal contraction and sediment loading. By correcting the well data for the effects of sedimentary loading it has, therefore, been possible to estimate the form of the thermal contraction and how it may vary across a margin.

Most modelling studies now assume that thermal contraction at passive margins arises from heating and thinning of the crust and lithosphere at the time of initial rifting^{22,23}. The main differences between the various models is the manner by which the heating and thinning occurs. Sleep²² proposed that the thinning was caused by uplift and subaerial erosion while McKenzie²³ proposed that it was caused by a passive, uniform extension of the crust and lithosphere.

There is good observational evidence for crustal extension during the early rifting history of passive margins, particularly at the sediment-starved margins of the eastern Atlantic and portions of the margin off eastern North America. For example, de Charpal *et al.*²⁴ have mapped listric faults and tilted fault blocks in northern Biscay, and Given²⁵ and Schlee²⁶ have mapped graben systems off Nova Scotia and New England. The amount of extension cannot easily be estimated from fault geometry, but seismic refraction data are consistent with a stretching factor²³ of $\beta = 2-3$ over distances of 100–200 km in northern Biscay^{27,28}.

The stretching model²³ has also been applied to the thickly sedimented margins of the western Mediterranean off southern France²¹ and portions of the margin off eastern North America^{29,30}. The main difficulty is the lack of information at these margins on the thickness and depositional environment of the sediments formed during rifting and, the tectonic fabric of the underlying basement rocks. For example, the relative proportion of syn-rift to post-rift sediments, an important constraint in the stretching model³¹, is not precisely known at these margins. The available evidence^{29,30} suggest a stretching factor in the range $\beta = 1.5-6.0$. Unfortunately, there is too little seismic refraction data to constrain these estimates satisfactorily but geoid data are consistent with large amounts of stretching ($\beta = 3-4$) over distances of 100–200 km along portions of these margins (ref. 20 and M. S. Steckler, personal communication).

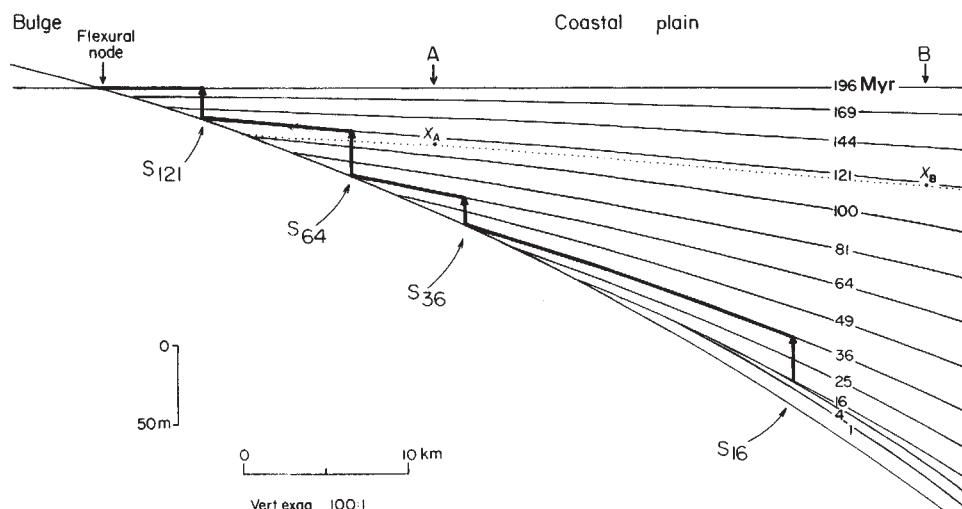
The model of McKenzie²³ therefore appears to give a general explanation of the available geological and geophysical data at passive margins. Some problems still remain, however, the most important of which are: the discrepancies between estimates of stretching based on fault geometry and deep crustal seismic refraction data²⁸, the occurrence of seaward-dipping reflectors of possible subaerial volcanic origin at some margins³² and the relative role of passive and active heating in the thermal evolution of a margin²¹.

Flexure

The response of the lithosphere to sediment loads at a passive margin has been modelled using either an Airy or flexure model of isostasy^{17,18}. Seismic reflection profiles indicate that active faulting accompanies the early stages of rifting²⁴⁻²⁶. This suggests that an Airy model, in which sediments are locally compensated, is most applicable early in margin evolution. The presence of gently dipping post-rift sediments and a broad coastal plain, however, suggests the flexure model becomes more important later in margin evolution.

The actual lithospheric response to sediment loads is difficult to determine at a passive margin because sediments are continually added to it during long periods of time. The best evidence has come from studies of seamounts and oceanic islands, as they formed during relatively short time periods (1–1.5 Myr). These studies show that the flexural rigidity and the equivalent elastic thickness of oceanic lithosphere, T_e , increases with the age of the lithosphere at the time it was loaded (Fig. 1). Loads formed on young oceanic lithosphere, such as the Walvis ridge, are associated with small values of T_e while loads formed on old oceanic lithosphere, such as the

Fig. 3 Coastal plain stratigraphy calculated using the thermal and mechanical model in Fig. 2. The model calculations assume $\beta = 3.0$ and $T_e = Z_{450}^\circ\text{C}$. Solid lines represent individual stratigraphical horizons at different times following rifting. The effects on the stratigraphy of compaction have not been included. The heavy solid lines illustrate the procedure used by Vail *et al.*⁷ and this study to estimate the vertical component of coastal onlap. The dotted line indicates the equilibrium shoreline where the rate of change of long-term sea-level fall⁹ equals the subsidence rate. Because the coastal plain sediments subside at a slower rate than long-term sea-level rises or falls, there is a tendency, depending on the rate of sediment influx, for transgression below the dotted line (enhancing the effects of flexure) and regression above the line (competing with flexure).



Hawaiian islands, are associated with large values. Figure 1 shows there is good general agreement between T_e and the depth, Z , to the 300–600 °C oceanic isotherms. Thus, flexure studies in the ocean basins suggest that as the oceanic lithosphere increases in age and cools, it becomes more rigid in its response to loads.

Because the lithosphere at passive margins is extensively heated during rifting^{22,23}, it should therefore become progressively more rigid in its response to sediment loads as it cools

following rifting. Sediments formed soon after rifting would be expected to load a relatively hot and weak lithosphere while later sediments would load a relatively cool and strong lithosphere. A flexure model, in which the rigidity of the lithosphere increases with time, explains certain tectonic-stratigraphical features of well sedimented passive margins such as an outer stratigraphical high, a hinge zone and a coastal plain in which younger sediments progressively onlap basement³³.

Thermal and mechanical models

Thermal and mechanical models have now been constructed^{15,16,34} for the stratigraphy of passive margins that combine the effects of thermal contraction and sedimentary loading. In these models, thermal contraction occurs following crustal and lithospheric extension at the time of rifting while sedimentary loading occurs by flexure of a progressively more rigid basement following rifting. Since T_e is a strong function of temperature (Fig. 1), thermal and mechanical effects can be coupled. Thus, T_e can be calculated as a function of time and position following rifting.

Figure 2 shows a simple model for the tectonic evolution of a passive margin that formed by crustal and lithospheric extension at the time of rifting. The region of extension is limited to beneath the shelf and slope and to have a magnitude ($\beta = 3$) and horizontal extent (175 km), similar to present day margins. Sediments are assumed to infill uniformly the cooling margin, maintaining a constant bathymetric profile with time. The model includes the effects of lateral heat conduction across the stretched region and flexure that varies as a function of time and position ($T_e = Z_{450}^\circ\text{C}$).

Figure 3 shows the stratigraphy predicted by the thermal and mechanical model at the edge of the margin. The solid lines indicate stratigraphical horizons at equal increments of the square root of time since rifting. Figure 3 shows that there is initially a significant onlap of sediments onto the basement, due to the abrupt transition in the stretching model from fault-controlled Airy-type subsidence to flexural controlled subsidence. Soon after rifting, the pattern of onlap is abruptly terminated because lateral heat flow from the stretched region to unstretched continental lithosphere causes the coastal plain to remain emergent^{15,16}. Beginning about 16 Myr after rifting, however, flexure overcomes the effects of lateral heat flow and younger sediments progressively onlap the basement, due to its increase in flexural rigidity with age.

The model in Fig. 3 is simplified as it assumes an abrupt increase in β across the hinge zone and a sediment influx that

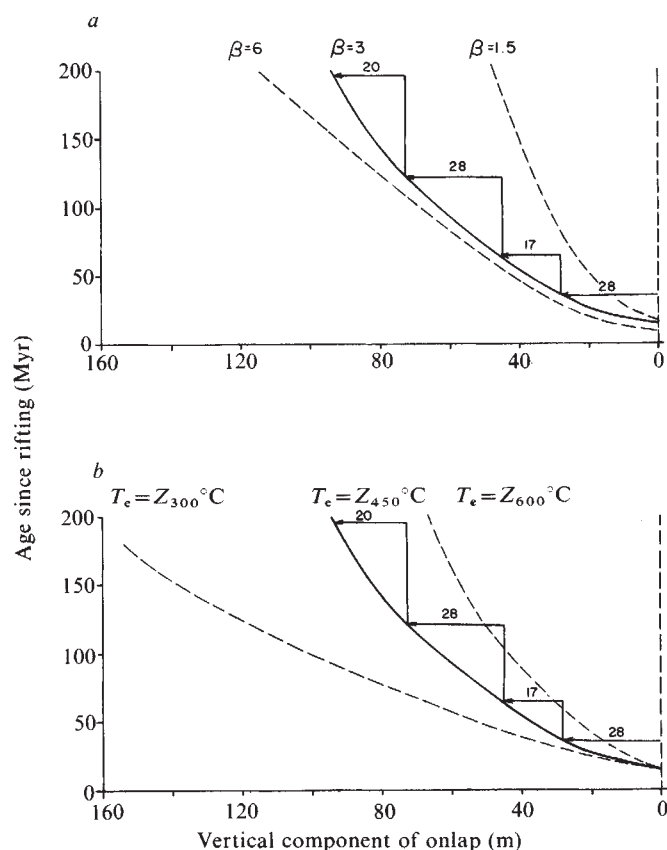


Fig. 4 The vertical component of coastal onlap estimated from the thermal and mechanical model in Figs 2 and 3. Solid lines indicate the individual measured increments of coastal onlap and the heavy solid lines indicate the smoothed change in onlap following rifting. *a*, $T_e = Z_{450}^\circ\text{C}$ and β variable. *b*, $\beta = 3.0$ and T_e variable.

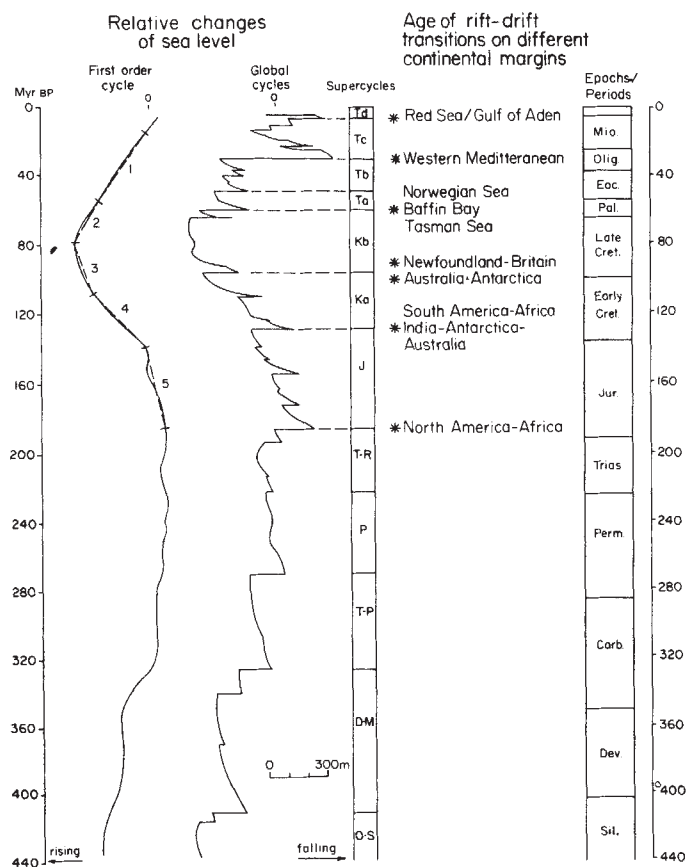


Fig. 5 Global cycles of relative changes of sea level since the Devonian⁶ compared with the age of the rift-drift transition at different continental margins⁴⁸. The numbered dashed lines on the 'first-order cycle' are construction lines used to estimate the rates of change of sea-level rise and fall in Fig. 6.

uniformly infills shelf, slope and rise regions. Although there is little information on how β varies across a hinge zone, it seems likely that it would change more smoothly than assumed in the model. An abrupt change in β maximizes the lateral effect of heat flow across the hinge zone so the coastal plain would remain emergent for a longer time following rifting. However, a more gradual transition in β across the hinge zone only slightly decreases the pattern of onlap in Fig. 3. The pattern of onlap would be expected to change if sediments by-passed shelf regions due, for example, to vigorous submarine erosion²⁶. A prolonged absence of shelf loading would cause the flexural node (Fig. 2b) to migrate seaward towards loaded slope and rise regions, resulting in offlap in the coastal plain. Changes in the pattern of sediment influx seem unlikely, however, to compete with the modelled onlap in Fig. 3 unless it, like flexure, varies systematically during margin evolution.

The model studies therefore suggest that coastal onlap is a characteristic feature of stratigraphical sequences at a cooling margin. This result is important since measurements of the amount of onlap at present day and ancient passive margins is the principal means by which Vail *et al.*^{6,7} estimate sea-level changes through time. The models in Figs 2 and 3 have been constructed assuming no sea-level changes through time. Thus, by measuring the amount of onlap in the models we can estimate the contributions of tectonics to stratigraphical sequences.

Vail *et al.*⁷ measured onlap by estimating the 'coastal aggradation', or 'vertical component of onlap', in a stratigraphical sequence. Beginning with the lowest point of onlap in a sequence, they successively summed each increment of onlap until the highest point of onlap is reached. Figure 3 illustrates, on the modelled stratigraphy, the procedure used by Vail *et*

*al.*⁷. Starting with S₁₆, the lowest point of onlap in the sequence, the first increment of onlap is 28 m and the time interval is 16–36 Myr. Successive increments of 17, 28 and 20 m give a total amount of onlap for the sequence of 93 m. Figure 4 shows the largest amount of onlap (100–160 m) occurs for the greatest amount of stretching and weakest plate, while the smallest amount (45–65 m) occurs for the least stretching and strongest plate. The form of the onlap is similar in each model, generally following the shape of an exponential curve, with an initial rapid increase followed by a more gentle increase.

Although they did not detail their method, Vail *et al.*⁷ used coastal onlap to estimate sea-level rise through time. They used a modal average of three or more correlative cycles of onlap from different continents to construct a global sea-level curve for the Phanerozoic. But, as Fig. 4 shows, coastal onlap is a characteristic feature of the tectonic evolution of a cooling passive margin and does not require sea-level changes to produce it. Therefore, if the model predictions are correct, some correlation should exist between the beginning of major cycles of onlap in the Vail *et al.*⁶ curve and the age of the rift-drift transition in passive margins, formed as a result of continental break-up.

Figure 5 shows there is an excellent visual correlation between the beginning of the supercycles of Vail *et al.*⁶ and the age of the rift-drift transition at margins formed by the breakup of the Pangea supercontinent. There is a striking similarity between the form of the modelled onlap and individual supercycles (Fig. 5). This similarity suggests, therefore, that major portions of the Vail *et al.*⁶ curve may have a tectonic, rather than eustatic, control.

Vail *et al.*⁶, however, terminate their supercycles by rapid sea-level falls (Fig. 5). The tectonic models predict regressive sequences early in the development of a margin (Fig. 3) but, they do not predict them at higher levels in a stratigraphical sequence. Therefore, if the magnitudes of the sea-level falls deduced by Vail *et al.*⁶ are correct then, factors other than flexure must be invoked to explain them.

There is some doubt that Vail *et al.*⁶ have correctly estimated the amount of sea-level fall from stratigraphical sequences^{14,35}. The main problem is that they determine sea-level fall by measuring the vertical component of onlap between the highest point of onlap in an underlying sequence directly to the lowest point of onlap in an overlying sequence. Because the record is largely removed by erosion during a sea-level fall, they cannot use the 'incremental method' that was used to measure onlap. Thus, the effects of tectonics during the sea-level fall are not satisfactorily accounted for.

There is good evidence³⁶, however, for major periods of regression in the geological record, although their origin is in some doubt. For example, Grasty³⁷ has related major regressive events to crustal thickening associated with orogeny while Ager³⁶ has related them to long-term changes in global sea level, due to changes in mid-ocean ridge crest volumes.

The effects of long-term changes in sea level^{9,6} on the modelled stratigraphy are illustrated in Fig. 6. The open triangles (Fig. 6) indicate the age since rifting when the rate of sea-level fall equals the rate of model subsidence and the shoreline would stabilize. For small times following rifting the seas would rise faster than the model subsides, causing a transgression, while for longer times the seas would fall faster, causing a regression. The actual effect of long-term sea-level changes depends, however, on the relative rates of sediment influx and subsidence². For the modelled stratigraphy in Fig. 3, a transgressive sequence would be expected to develop soon after rifting, enhancing the pattern of onlap produced by flexure, while for later times a regressive sequence would develop, competing with flexure. The timing of the resulting seaward shift in onlap would depend on position, but for the model in Fig. 3 it occurs about 112–117 Myr following rifting.

The model predictions are difficult to compare with the Vail *et al.*⁶ curve as this curve represents the summation of several shifts in the pattern of onlap from different positions in widely

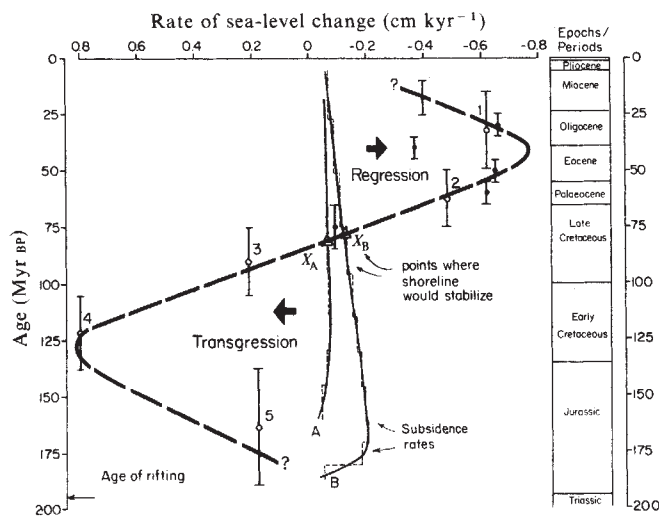


Fig. 6 Comparison of the rate of change of sea level to the subsidence rate at two points (A, B) in the thermal and mechanical model in Fig. 3. The rate of change of sea level is based on the long-term sea-level curve of Pitman⁹ and the first-order cycle of Vail *et al.*⁶. ●, Based on Table 2 of ref. 9; ○, a straight line fit to the first-order cycle of Vail *et al.*⁶ (Fig. 5). △, The time in Myr BP that the rate of sea-level fall equals the rate of subsidence and the shoreline, in the absence of other effects, would be in equilibrium.

spaced margins. There is evidence though, from published geological cross-sections of the US Atlantic coastal plain³⁸, of a progressive onlap of Jurassic to early Cretaceous sediments onto basement that is terminated by a seaward shift in the overall pattern of onlap about 120 Myr after rifting. Thus, the model in Fig. 3 seems to agree with the overall stratigraphy of this relatively old margin.

The tectonic models are still unable, however, to explain the widespread rapid short-term falls of sea level in the Vail *et al.*⁶ curve. Thus, without a satisfactory mechanism, the origin of these falls remains in doubt.

Discussion

The model studies suggest that tectonics is an important control on the development of stratigraphical sequences at passive margins. Flexure produces patterns of onlap, remarkably similar in form to those used by Vail *et al.*⁷ to infer sea-level rise. Although Vail *et al.*⁷ attempted to remove tectonic effects by summing increments of coastal onlap in a sequence, they did not correct for flexural effects that vary as a function of time and position. They considered only a limited role for tectonics that was generally equivalent to assuming sedimentary loading occurs by flexure of a lithosphere of uniform flexural rigidity.

prominent pattern of onlap for each of these periods which they interpreted as a sea-level rise. The increase in width of the Michigan basin during Devonian⁴⁵ can be explained⁴⁶, however, by an increase in flexural rigidity of the lithosphere with age, and does not require sea-level changes to produce it. Furthermore, the apparent progressive onlap of sediments onto the western and eastern margins of the North American craton during the Cambrian⁴⁷ can be explained by flexure, although the large horizontal extent of the Albertan may require long-term sea-level changes in addition.

This is not to imply that a completely satisfactory model exists yet for the tectonic evolution of sedimentary basins in present day and ancient passive margins. There is too little seismic reflection and refraction data at present day margins that satisfactorily constrains the deep crustal structure implied

by the thermal models. The occurrence of coastal onlap at a passive margin, however, is the consequence of the cooling of the lithosphere following rifting and does not depend on a particular type of thermal model. The actual separation of tectonic effects from stratigraphical sequences remains a difficult problem that will require careful studies of seismic, geological and palaeontological data from localities in widely separated margins. The problem is important for future consideration, however, as it has major implications for tectonics, lithospheric mechanics, correlative stratigraphy and the thermal history of the Earth.

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Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus *ras* gene

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Examination of homologies between retroviral oncogenes and transforming sequences defined by transfection reveals that the human bladder carcinoma (EJ) oncogene is homologous to the Harvey sarcoma virus oncogene (ras). Structural analysis limits the region of homology to a 3.0-kilobase SacI fragment of the EJ oncogene. Both EJ and ras DNA probes detect similar transcripts in transfectants derived from bladder carcinoma cell lines.

TWO groups of cellular oncogenes have been discovered during the past decade. The first consists of genes that were characterized by virtue of their association with retroviruses. The prototype of this class is the *src* gene of avian sarcoma virus. Several experiments have indicated that this gene was acquired from the chicken genome by an avian retrovirus, and has been exploited by the chimaeric virus to transform cells^{1,2}. Once incorporated into the viral genome, expression of the *src* gene is driven by viral controlling elements and is no longer responsive to control mechanisms that governed its expression while in the cellular chromosome. In addition to the *src* gene, this group includes at least 12 other gene sequences, each associated with a different chimaeric retrovirus^{3,4}. These genes are conserved over great evolutionary distances^{2,5} implying that they mediate essential cellular or organismic functions.

A second class of cellular transforming genes has been detected by the experimental route of DNA transfection. Recent reports have indicated that the DNAs of some non-virally induced tumour cell lines can induce transformation when applied to mouse fibroblast monolayers. These tumour cell lines are derived from chemically induced animal tumours⁶⁻⁹ and from human tumours of spontaneous origin^{7,10-12}.

The two classes of oncogenes have many properties in common, the most striking of which is the apparent origin of both types of genes from normally benign, cellular genetic elements. Because of this and other parallels, we undertook a search to determine whether the two groups of genes shared any members in common. Such overlap would have far-reaching consequences for our understanding of the mechanisms of viral and non-viral carcinogenesis.

Table 1 Oncogene probes

Oncogene‡	Related virus or tumour	Clone	Sequence complexity	Source	Ref.
<i>v-abl</i>	Murine Abelson leukaemia virus	pAblsub9	3.0 kbp	J. Y. J. Wang and D. Baltimore	*
<i>v-erb</i>	Avian erythroblastosis virus	PAE- <i>PvuII</i>	2.5 kbp	T. Gonda and J. M. Bishop	28
<i>v-ST-fes</i>	Snyder-Theilen feline sarcoma virus	PST-3	0.6 kbp	C. Sherr	29
<i>c-Ha-ras1</i> (rat)	Murine Harvey sarcoma virus	LHXB-3	2.3 kbp	R. Ellis and E. Scolnick	30
<i>v-Ha-ras</i>	Murine Harvey sarcoma virus	BS9	0.45 kbp	R. Ellis and E. Scolnick	21
<i>c-mos</i> (Human)	Murine Moloney sarcoma virus	pHM1	2.75 kbp	G. Vande Woude	†
<i>v-myc</i>	Avian myelocytomatosis virus	puMyC3- <i>Pst</i>	1.5 kbp	T. Gonda and J. M. Bishop	31
<i>v-src</i>	Avian sarcoma virus	SRA-2	0.8 kbp	T. Gonda and J. M. Bishop	32
	Human EJ bladder carcinoma	pEJ6.6	6.6 kbp	C. Shih	18

* J. Y. J. Wang and D. Baltimore, in preparation. † G. Vande Woude, personal communication. ‡ *v-* indicates viral gene; *c-* indicates cellular gene.

Detection of homologies by nucleic acid hybridization

The search for relatedness between the two groups of genes depended on detection of nucleic acid sequence homologies between individual members of each group. One group consisted of a series of seven retrovirus-associated *onc* genes known to be unrelated or only distantly related to one another⁴. The other group studied was a collection of seven tumour oncogenes that had been defined by transfection^{7,10}. Nucleic acid sequence probes have been derived for the retrovirus-associated genes, whereas only a few of the transfection-derived genes have been isolated in the form of molecular clones. We therefore used the virus-derived *onc* probes to survey the DNAs of cells which had acquired, via transfection, copies of the second class of genes. The survey was performed using the Southern gel-filter transfer procedure¹³. Virus-derived *onc* probes were from several sources (see Table 1).

We used each retrovirus-related *onc* probe to search for novel cross-reacting fragments in each of several transfectants. For example, an analysis using the *v-abl* probe is shown in Fig. 1: lane *a* represents the endogenous mouse sequences detected

in the DNA of untransfected NIH 3T3 cells by the *v-abl* sequence probe. These 'background' bands of 28, 10 and 6 kilobases (kb) were also found in the DNAs of all transfectants (Fig. 1, lanes *b*, *d-g* and *i*). Figure 1*c* shows detection of the human homologue of the *v-abl* probe; lanes *d-g* show an analysis of DNAs of mouse cells transfected with four different human oncogenes. None of the DNAs contains any fragments beyond those present in the untransfected mouse control (lane *a*). We concluded that none of these transfected cells acquired the human homologue of the *v-abl* sequence. From a similar analysis in lanes *h* and *i*, we concluded that the rabbit bladder carcinoma oncogene is also not related to the *abl* gene. A more equivocal interpretation came from analysis of the DNA of a mouse cell transfected with a mouse fibroblast oncogene (Fig. 1*a, b*). Due to the lack of species-specific fragment markers, we were unable to rule out the identity of the *abl* and fibroblast oncogene. However, knowledge of their restriction enzyme cleavage sites excludes identity^{14,15}.

Figure 2 shows a further Southern blot analysis of DNAs that were prepared from several cell lines derived by transfection of NIH 3T3 cells with DNAs of human tumour cell lines. The probe used was the rat cellular homologue of Harvey sarcoma virus, *c-Ha-ras1*, given by R. Ellis and E. Scolnick^{16,17}. A novel fragment of 9.5 kb was found in the DNA of a mouse cell transfected with DNA of the EJ human bladder carcinoma cell line (Fig. 2*c*). In contrast, no novel fragments were present in the transfectants derived from other tumour cell lines (Fig. 2*d-g*).

Of the seven *onc* probes used in this survey, only one detected the presence of novel DNA fragments in transfection-derived cell lines. The results of this comparative oncogene survey are summarized in Table 2. Below we consider the relationship between the *c-Ha-ras1* gene and the human bladder carcinoma gene suggested by Fig. 2.

Homology of the two oncogene DNAs

A series of tests was performed to further substantiate the relationship between the EJ bladder oncogene and the *c-ras* oncogene. Figure 3*A* shows that nine cell lines derived by transfection of EJ bladder carcinoma DNA have all acquired novel DNA fragments reactive with the *c-Ha-ras1* probe. DNA from untransfected mouse cells (Fig. 3*A*, lane *a*) does not exhibit any of these novel fragments. The *Bam*HI-digested DNAs of the various transfectants exhibit differently sized novel fragments because of rearrangements occurring during the transfection process. Note that the oncogene of the T24 human bladder carcinoma is closely related to that of the EJ bladder carcinoma^{18,19}. Figure 3*A*, lane *m*, indicates that a mouse transfectant carrying the T24 oncogene also contains in its DNA a novel acquired fragment reactive with the probe.

To further define the linkage between the EJ oncogene and the *c-Ha-ras1* homologous sequences, we analysed the DNAs of three EJ transfectants derived by transfer of *Bam*HI-cleaved DNA (Fig. 3*B*). The donors of these DNAs were secondary transfectants derived previously by two serial passages of the bladder carcinoma oncogene. As endonuclease *Bam*HI does

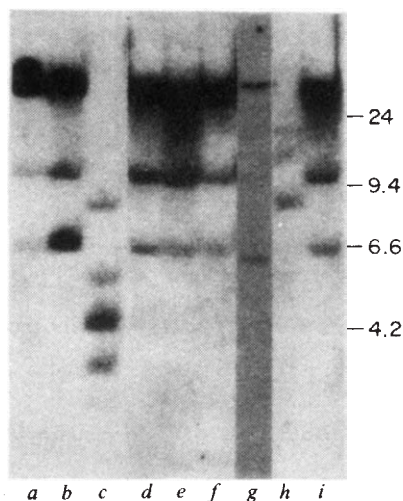


Fig. 1 Southern blot analysis of digested cellular DNAs from various transfectants probed with *v-abl* specific probe (see Table 1). 10 µg of each DNA were digested with endonuclease *Eco*RI, fractionated by electrophoresis through a 1% agarose gel and transferred to nitrocellulose paper¹³. The filters were incubated with 5 × 10⁶ c.p.m. of nick-translated ³²P-labelled Abelson virus specific probe. The DNAs analysed were from the following cell lines: *a*, NIH 3T3; *b*, Y5-1; *c*, HeLa; *d*, A5-2; *e*, SH-1-1; *f*, SW-2-1; *g*, EJ-6-1; *h*, rabbit embryo fibroblast; *i*, RBC-1. Lanes *a-f* and *h, i* are from one filter; *g* is from a different filter. The transfected cell lines are described in Table 2. Migration of *Hind*III-digested λ DNA fragments are shown at the right (in kilobase pairs, kbp).

Table 2 Comparison of transfected oncogenes with retroviral *onc* probes

<i>onc</i> Probes	Transfected cell lines							
	Y5-1-1	EJ-6-1	A5-2	SH-1-1	SW-2-1	HL60-1-9	B104-1-1	RBC-1
<i>v-abl</i>	*	—	—	—	—	—	NT	—
<i>v-erb</i>	*	—	—	—	—	—	NT	NT
<i>v-fes</i>	*	—	—	—	—	—	—	NT
c-Ha- <i>ras1</i> (rat)	*	+	—	—	—	—	—	NT
<i>c-mos</i> (Human)	*	—	NT	NT	—	—	NT	NT
<i>v-myc</i>	*	—	—	—	—	—	—	NT
<i>v-src</i>	*	—	—	NT	—	—	NT	—

The oncogene probes shown are described in Table 1. The transfected cell lines listed are of the following origins: Y5-1-1 is derived from two serial passages of the oncogenic DNA from a 3-methylcholanthrene-induced mouse fibroblast cell line, MCA-16 (refs 6, 33); EJ-6-1 is a secondary transfected cell line derived from DNA of a human bladder carcinoma cell line, EJ⁷; A5-2 is a primary transfected cell line derived from human lung carcinoma cell line, A549 (ref. 34); SH-1-1 is a secondary transfected cell line derived from DNA from human neuroblastoma cell line, SK-N-SH (J. Føgh, personal communication); SW-2-1 is a secondary transfected cell line derived from human colon carcinoma cell line, SW-480 (refs 10, 35); HL60-1-9 is a secondary transfected cell line derived from human leukaemia cell line, HL60 (refs 10, 36); B104-1-1 is a secondary transfected cell line derived from rat neuroblastoma cell line, B104 (refs 7, 37); RBC-1 is a primary transfected cell line derived from rabbit bladder carcinoma cell line, RBC^{7,38}. + Indicates hybridization of the probe to novel bands in addition to hybridization with NIH 3T3 cellular sequences; — indicates a well controlled negative correlation. * Apparent negative correlation that is not based on species-specific DNA fragment sizes that distinguish donor DNAs from resident chromosomal DNAs of the recipient cells. NT, not tested.

not inactivate the EJ oncogene¹⁸, the transfection of *Bam*HI-cleaved secondary DNA ensured that almost the only human fragment present in resulting tertiary transfectants was the 6.6-kb *Bam*HI fragment bearing the EJ oncogene. The three DNAs of the tertiary transfectants were analysed after *Eco*RI cleavage as it was thought they might have lost some *Bam*HI sites during the second transfection. The DNAs of the three transfectants (Fig. 3B, lanes a–c) all showed acquired fragments reactive with the c-Ha-*ras1* probe. This demonstrated that the linkage between the EJ oncogene and the c-Ha-*ras1* homologous sequences could not be broken by *Bam*HI cleavage.

A further comparison between the genes depended on the fact that the EJ bladder carcinoma oncogene was one that we have recently isolated as a molecular clone¹⁸. The EJ human bladder oncogene has been cloned as a biologically active *Eco*RI fragment of 16 kb carried by a Charon 4A λ phage vector and termed Φ 631. A biologically active 6.6-kb *Bam*HI fragment subclone has been inserted into plasmid vector pBR322 and termed pEJ6.6. All endonucleases shown to cleave within this 6.6 kb insert (Fig. 4) inactivate the focus-inducing activity of this DNA (ref. 18 and C. Shih, unpublished results).

Using the EJ 6.6-kb *Bam*HI fragment and the c-Ha-*ras1* oncogene clone as sequence probes, we analysed the DNA fragments homologous to these genes in normal human DNA. Both probes detected a 6.6-kb *Bam*HI fragment (Fig. 3A, C, lanes b) and a 23-kb *Eco*RI fragment (Fig. 3B, D, lanes b) in

human DNA (see also ref. 20). Furthermore, the EJ bladder oncogene probe detected the same novel fragments in transfected mouse lines (Fig. 3C, lanes c–k) that were previously detected using the c-Ha-*ras1* probe (Fig. 3A, lanes c–k).

Endonuclease-cleaved Φ 631 DNA was immobilized on a cellulose nitrate filter and probed with the c-Ha-*ras1* sequences. Figure 4a indicates that homology between c-Ha-*ras1* and the EJ clone is limited to the 6.6-kb *Bam*HI fragment of the bladder carcinoma oncogene; lane b further reduces the domain of homology between the two oncogenes to a 3.0-kb *Sac*I fragment within the 6.6-kb *Bam*HI fragment. Figure 4f–i shows a similar experiment to that of lanes b–e but in this case BS-9, a v-Ha-*ras* probe, was used (provided by Drs D. Lowy and E. Scolnick). BS-9 is a *ras* specific subclone of the Harvey sarcoma virus genome²¹ and is ~450 base pairs (bp) long. This probe includes the 5' half of the v-*ras* gene. Comparison of left and right panels of Fig. 4 confirms that the viral probe and c-Ha-*ras1* cross-hybridize with identical fragments of the EJ oncogene DNA. Double digests (Fig. 4g, i) with *Sac*I+*Kpn*I or *Sac*I+*Xba*I indicate that the v-Ha-*ras* homology straddles the *Kpn*I and *Xba*I cleavage sites indicated at the top of Fig. 4. Most of the reactivity of the c-Ha-*ras1* probe lies in the larger of the two fragments created by these digests (Fig. 4c, e). The deduced alignments between the EJ oncogene and the v-Ha-*ras* and c-Ha-*ras1* probes are shown at the top of Fig. 4. The direction of transcription, deduced from the results of the present study and previous data^{16,17}, is from right to left on the map.

Taken together, these results indicate that the EJ bladder oncogene is closely linked to the human homologue of the rat c-Ha-*ras1* sequences. Although the limits of the c-Ha-*ras1* structural sequences have been well defined^{16,17,20}, the corresponding sequences of the EJ gene have not yet been mapped. Thus, the data above cannot exclude the possibility that the two genetic elements were adjacent to one another rather than congruent.

Analysis of transcripts homologous to the clones

The transcripts encoded by these genes were analysed to further establish their relationship to one another. We examined the RNAs of transfected cells for molecules reactive with the two oncogene probes. As shown in Fig. 5, the two probes each detected transcripts of 1.2 and 5.1 kb in both the parental tumour cell line and in EJ- and T24-transfected mouse cell lines (see also ref. 22). These transcripts were not detected in untransfected NIH 3T3 cells. Thus, introduction of the EJ oncogene into mouse cells results in synthesis of RNAs that are homologous with the rat c-Ha-*ras1* gene. As discussed below, these data support a congruency between the functionally active region of the EJ gene and that of the c-*ras* gene.

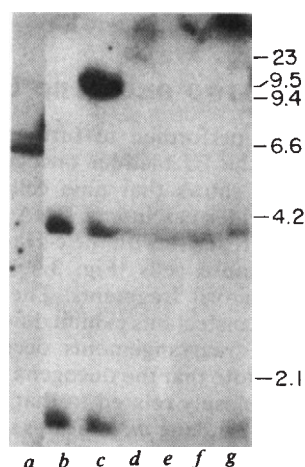


Fig. 2 Southern blot analysis of DNA from transfected cells with rat cellular *ras* probe (c-Ha-*ras1*). ³²P-labelled c-Ha-*ras1* DNA was prepared as described in Fig. 1 legend and incubated with a filter carrying *Bam*HI-digested DNAs from the following cell lines: a, HeLa; b, NIH 3T3; c, EJ-6-1; d, A5-2; e, SH-1-1; f, SW-2-1; g, HL-60-9. Lanes b–g are from the same filter. The cell lines are described in Table 2.

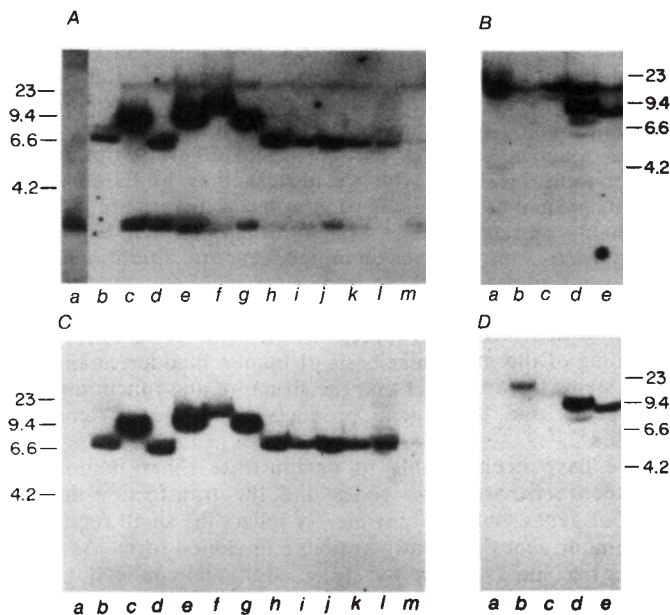


Fig. 3 Analysis of DNAs from EJ and T24 transfectants using the c-Ha-ras1 and EJ oncogene probes. DNAs were digested with endonuclease *Bam*HI (A, C) or *Eco*RI (B, D) and analysed as described in Fig. 1 legend. The filters shown A and B were incubated with 5×10^6 c.p.m. of c-Ha-ras1 DNA probe (2×10^8 c.p.m. μg^{-1}) and exposed for autoradiography for 16 h. The adsorbed ^{32}P -labelled probe was removed from the filters by washing in 0.1 M NaOH, 0.5 M NaCl, 1.0 mM EDTA. The washed filters were exposed to film for 48 h to verify that all the radioactive signal had been removed (not shown). The filters were then incubated with 5×10^6 c.p.m. of nick-translated pEJ6.6 DNA (6×10^7 c.p.m. μg^{-1}). C and D represent autoradiography after 16 h. The DNAs analysed in panels A and C were from the following cell lines: a, NIH 3T3; b, HeLa; c, EJ-6-1; d, EJ-2-R1; e, EJ-2-R5; f, E-4-R4-B; g, EJ-6-1; h, EJ-6-2-R; i, EJ-6-3; j, EJ-1-2; k, EJ-3-2; l, T-24 human cells¹⁹; m, T24-8-5. B and D display DNAs from: a, NIH cells; b, HeLa cells; c, EJ-6-2 (*Bam*)-1 cells; d, EJ-6-2(*Bam*)-2 cells; e, EJ-4(*Bam*)-1 cells. In A and C, lanes c-f and h-k represent DNA from cell lines that were derived from independent serial transfections of the EJ tumour cell oncogene; lanes g and l are duplicates. Lane 1 contains DNA from human bladder carcinoma cell line T24. Lane m contains DNA from a secondary transfectant of T24. Lanes c-e of B and D show an analysis of DNAs from tertiary transfectants induced by exposure to *Bam*HI-cleaved secondary transfectant DNA.

Discussion

The present data strongly suggest an evolutionary homology between the EJ human bladder carcinoma oncogene and the rat c-Ha-ras1 gene. We now consider the experimental basis for this conclusion and its implications.

The relatedness between the EJ and c-Ha-ras1 genes was first noted when we demonstrated that a cell acquiring the EJ oncogene also carried a novel DNA fragment reactive with the c-Ha-ras1 probe. As the linkage between the c-Ha-ras1 homologous sequence and the EJ oncogene was not broken by endonuclease *Bam*HI, we concluded that the two genetic elements lay within the same 6.6-kb *Bam*HI-generated fragment. These data alone were consistent with the two elements being either physically adjacent or congruent with one another. To resolve this ambiguity, we analysed the transcripts encoded by the two oncogenes. The EJ parental tumour and its derived transfectants, all express 5.1- and 1.2-kb transcripts that react with the oncogene probe. The fact that these transcripts are also present in cells transfected with *Bam*HI-cleaved DNA (data not shown) implies that the entire transcriptional unit of the 5.1-kb RNA is found within the confines of the 6.6-kb *Bam*HI-generated fragment (Fig. 3B, D).

It could be argued that the transcripts detected in transfected mouse cells are of murine origin. In this case, their synthesis would be induced indirectly in the mouse cells by the acquired human oncogene rather than being encoded directly by the human gene. We consider this unlikely, as the rat c-Ha-ras1 probe used had a three-fold higher specific radioactivity than the human EJ probe, but yielded a 3-4-fold lower signal intensity on autoradiography (Fig. 5). This must reflect the relatively lower affinity of the rat probe for human transcripts present in the EJ transfectants. Thus, we conclude that the two probes both detect RNAs transcribed largely, if not entirely, from the human EJ oncogene template.

A similar, if not identical, pair of transcripts has been found by colleagues working with the c-Ha-ras1 gene and its RNAs²². As both the EJ and c-Ha-ras1 homologous 5.1-kb transcriptional units lie within the 6.6-kb *Bam*HI fragment, we conclude that the two genes are congruent with one another rather than adjacent. Consistent with this structural homology is a functional analogy in that both genes are able to induce fibroblast transformation.

The *ras* genes encode proteins of molecular weight 21,000 (ref. 23). Immunoprecipitation of metabolically labelled lysates of transfected cells has detected a protein of this size (R. Finkelstein and R.A.W., unpublished observations). However, the association of this protein with the bladder oncogene will only be well established after its detailed peptide structure has been analysed. Knowledge of the function of this protein may elucidate the important steps in human bladder carcinogenesis.

This p21 polypeptide represents a strong candidate for the protein mediating transformation of a human tumour cell. It is

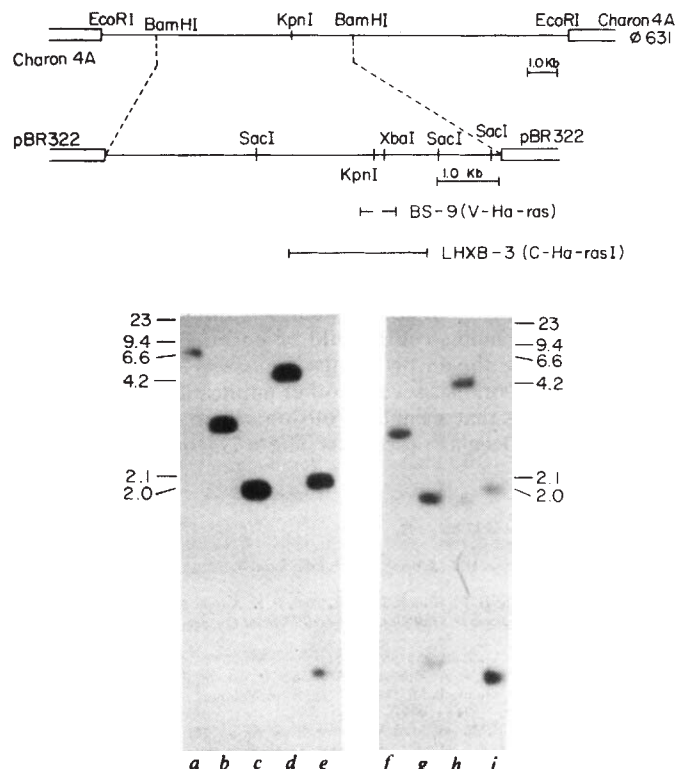


Fig. 4 Alignment of c-Ha-ras1 and of v-Ha-ras (BS-9) with the physical map of the EJ oncogene. DNA from the EJ-Charon 4A clone $\Phi 631$ was digested with several restriction enzymes. DNA (0.5 μg) was loaded onto each lane before electrophoresis and blot transfer. Identical nitrocellulose filters were prepared and incubated with ^{32}P -labelled DNA of c-Ha-ras1 (lanes a-e) or with HaSV subclone BS-9 (lanes f-i). $\Phi 631$ DNA was cleaved with *Bam*HI (a); *Bam*HI + *Sac*I (b, f); *Sac*I + *Kpn*I (c, g); *Bam*HI + *Xba*I (d, h); *Sac*I + *Xba*I (e, i). The alignment shown is accurate to within 200 nucleotides.

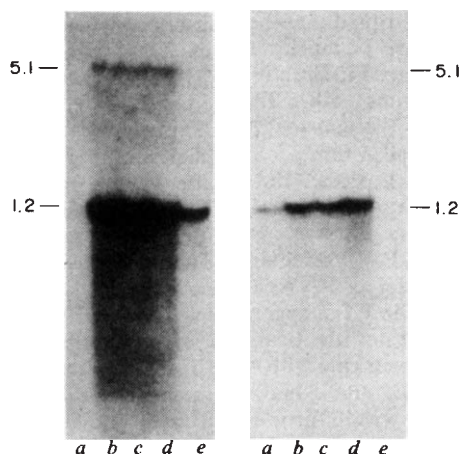


Fig. 5 Cellular polyadenylated RNAs analysed using pEJ6.6 and c-Ha-ras1 ^{32}P -labelled probes. In the left-hand panel, nick-translated c-Ha-ras1 DNA (2×10^8 c.p.m. μg^{-1}) was used to probe RNA isolated from the following cell lines: a, NIH 3T3; b, T24-8-1 (a secondary transfectant derived from T24 human cell line); c, EJ-4(R1)-2; d, EJ-6-2; e, EJ human bladder. In the right-hand panel, nick-translated pEJ6.6 (6.6×10^7 c.p.m. μg^{-1}) was used to probe the following cell lines: a, EJ; b, EJ-6-2; c, EJ-4(R1)-2; d, T24-8-1; e, NIH 3T3. The polyadenylated RNAs were prepared by the technique of Varmus *et al.*⁴⁰. The RNA was then fractionated by electrophoresis through formaldehyde-containing gels and transferred to nitrocellulose (B. Seed and D. Goldberg, in preparation). ^{32}P -labelled probes, prepared as described in Fig. 1 legend, were annealed to the immobilized RNAs⁴¹. Bands which represent sequences homologous to the probes were visualized by autoradiography. Molecular weights were determined by comparison with markers obtained from *in vitro* run-off transcription of the adenovirus late promoter⁴² and are shown in kilobases.

one of the first proteins implicated directly in the oncogenic conversion of a cell following its transformation by non-viral agents, and has been localized at the inner surface of the plasma membrane in Harvey sarcoma virus (HaSV)-transformed cells²⁴. If this localization applies also to the EJ bladder carcinoma cells, then the transforming protein of these cells, the p21, should not display extracellular antigenic determinants. In this case, any tumour-specific surface antigens displayed by the bladder carcinoma cell should be encoded by genetic elements other than the oncogene itself.

The present work has several other implications. Perhaps the most apparent is that a single proto-oncogene can be activated by different molecular processes. The c-Ha-ras1 oncogene of

the rat became activated via its affiliation with retrovirus sequences, forming the chimaeric Harvey sarcoma virus^{21,25}. As demonstrated in the accompanying article²⁶, the human c-Ha-ras1 gene is also capable of oncogenic activation after it becomes linked *in vitro* to retrovirus promoter sequences. The mode of activation of the EJ oncogene is different, but not yet understood. Presently evidence suggests that the EJ oncogene and its normal human allelic counterpart sequence are indistinguishable by restriction enzyme site mapping¹⁸. It is possible that its activation depends on minor structural alterations, such as point mutations.

The relatedness between the EJ oncogene and that of a transforming retrovirus represents an advance in our understanding of the molecular basis of human bladder carcinoma. This stems from the fact that the structure and function of the *ras* genes and their gene products have been extensively studied^{16-18,20-23}.

We have been unable to demonstrate other homologies between retrovirus *onc* genes and the transfection-derived tumour genes, but this may merely reflect the small repertoire of tumour genes presently available in cloned form. As other genes become available for study, additional connections will probably be found.

Two paradoxes seem to be raised by the unexpected association of a rat sarcoma oncogene with a human bladder oncogene. First, this work implies the ability of the c-Ha-ras1 gene to act in unrelated tissue environments. The rat gene, when carried in Harvey sarcoma virus, can induce sarcomas and erythroleukaemias²⁷ while its human counterpart is now implicated in the genesis of bladder carcinomas^{10-12,18,19}. We consider it possible that the *ras* oncogene of either species is capable of transforming a wide range of target tissues, only a small portion of which has been studied experimentally.

Second, we have suggested that the precursor of the EJ oncogene, now identified as c-Ha-ras1, represents a preferred target for activation during bladder carcinogenesis¹⁸. It seems unlikely that the bladder urothelium was the site of acquisition of the *ras* gene during the events that led to the creation of the chimaeric HaSV genome. The two routes of oncogene activation must involve different molecular mechanisms which probably occur at different sites in the organism. Each mode of activation may be favoured by different predisposing factors present in different tissues. For example, in the bladder, the c-Ha-ras1 gene may be in a configuration particularly susceptible to mutational activation whereas in certain other tissues it may be expressed in a manner favouring the recombinational events that lead to creation of chimaeric retroviruses.

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Tumorigenic transformation of mammalian cells induced by a normal human gene homologous to the oncogene of Harvey murine sarcoma virus

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A normal human gene homologous to the p21 ras oncogene of Harvey murine sarcoma virus induced oncogenic transformation and high p21 ras levels in murine fibroblasts when this gene was ligated to a control element (the long terminal repeat) from a murine or feline retrovirus. These results indicate that high levels of a gene product encoded by a normal human oncogene can induce tumorigenic transformation.

TRANSFORMING retroviruses have been isolated from diverse sources, including birds, rodents and non-human primates (for review see ref. 1). The *in vivo* tumorigenicity of these viruses has been correlated with their ability to induce focal transformation of tissue culture cells. These infected cells are fully transformed by the criteria of anchorage-independent growth *in vitro* and tumorigenicity in animals. As viral DNA genomes and subgenomic viral DNA fragments can induce similar transformation in mouse NIH 3T3 fibroblasts^{2,3}, this assay has been used to define the viral sequences required for focal transformation. Two components are essential: the viral transforming oncogene (*v-onc*) and the viral long terminal repeat (LTR), a control element which permits transcription of the *v-onc* gene^{4,5}.

More than ten *v-onc* genes have been described, each of which has been found to be transduced from a closely related, evolutionarily conserved, cellular oncogene (collectively called *c-onc*; reviewed in ref. 6). Using *v-onc* DNAs as probes and antibodies directed against *v-onc*-specified proteins, RNA and protein expression of some *c-onc* genes has been detected in normal cells. However, the level of *v-onc* expression in virally transformed cells is usually higher than that of *c-onc* expression in normal cells, suggesting that elevated levels of *c-onc* gene expression might induce oncogenic transformation. This prediction has recently been proven at least for two rodent *c-onc* genes: *c-mos*^{7,8}, the mouse sequences from which *v-mos* of Moloney murine sarcoma virus (Mo-MuSV) was derived, and *c-Ha-ras1* (ref. 9), a rat gene closely related to the transforming gene (*v-Ha-ras*) of the rat-derived Harvey (Ha) MuSV. The gene product of *v-Ha-ras* is a protein (p21) of molecular weight 21,000 (p21), and low levels of an immunologically cross-reacting non-viral p21 have been found in normal cells from various vertebrate species¹⁰. While *v-Ha-ras* contains no intervening sequences (IVS) within the p21 coding sequences, rat *c-Ha-ras1* has three IVS between its four exons. In contrast to most other *c-onc* genes, mouse *c-mos* does not contain intervening sequences, and transcription of this gene in normal cells has not been observed^{8,11}. Nonetheless, both rat *c-Ha-ras1* and mouse *c-mos* DNAs were capable of inducing cellular transformation of mouse NIH 3T3 cells, provided that a viral LTR was ligated upstream from the cellular *onc* gene. Transformation was associated with high RNA levels of *c-Ha-ras* or *c-mos*, respectively, and in the case of *c-Ha-ras*, high levels of p21.

Given the evolutionary conservation of *c-Ha-ras1* (refs 12, 13), we have sought to determine whether its counterpart in normal human cells has oncogenic potential similar to that of rat *c-Ha-ras1*. If the rat and human genes behaved similarly, it would suggest that the capacity of this oncogene to induce transformation was a property of this gene in any species. As part of our studies of *ras* genes, we have recently detected and cloned from normal human DNA four genes which share homology with *v-ras*¹³. The *ras* sequence in one of these human genes (called human *c-Ha-ras1*) was closely related to *v-Ha-ras* and had a structure very similar to that of rat *c-Ha-ras1*. We have now found that this human gene can induce oncogenic transformation of NIH 3T3 cells when an LTR is present upstream. The transformants form tumours in nude mice and

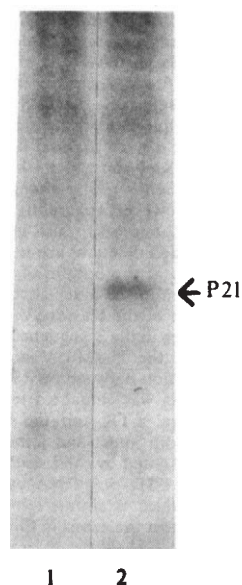


Fig. 1 Immunoprecipitation of endogenous p21 from normal human cells. WI-38 human fibroblasts were metabolically labelled with ³⁵S-methionine for 18 h and lysates were prepared and immunoprecipitated (5×10^6 c.p.m. of TCA-precipitable counts) by a monoclonal antibody as described elsewhere^{15,35}. Dissolved immunoprecipitates were then electrophoresed on a 10.5% SDS-polyacrylamide gel. Lane 1, anti- α -tubulin antibody; lane 2, *v-Ha-ras* p21-specific antibody (Y13-238). This monoclonal antibody was also used in Figs 4, 6.

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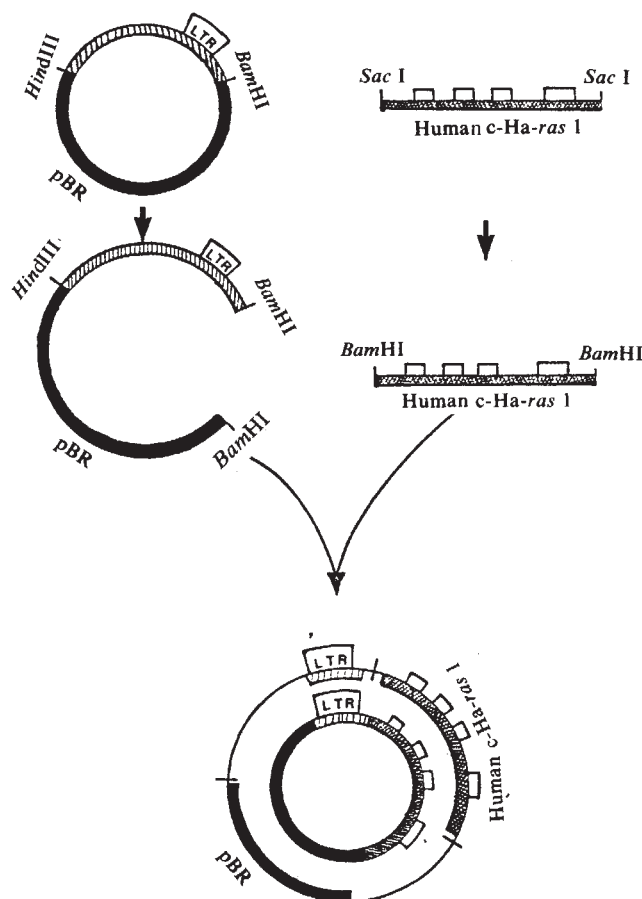


Fig. 2 Schematic representation of the construction of the Ha-MuSV LTR-human c-Ha-ras1 recombinant. To ligate an LTR upstream from the human c-Ha-ras1 gene, we constructed a chimaeric recombinant plasmid between the 2.9-kb *SacI* fragment of human c-Ha-ras1 and a pBR322 clone which already contained the Ha-MuSV LTR. This *SacI* fragment contains all the c-Ha-ras1 sequences which hybridized to v-Ha-ras¹². The pBR322 LTR clone chosen for this construction (clone P-14) contains a 2.5-kb *HindIII/BamHI* fragment of Ha-MuSV DNA; these sequences, which are derived from a circularly permuted molecule of Ha-MuSV¹⁸, span the region from a *HindIII* site in the 3' end of the Ha-MuSV DNA to the LTR and include 0.3 kb of 5' sequences beyond it to a *BamHI* site; they do not include v-Ha-ras sequences. To facilitate insertion of the c-Ha-ras1 *SacI* fragment at the unique *BamHI* site in clone P-14, an attempt was made, as shown in the figure, to add *BamHI* oligonucleotide linkers (Collaborative Research) to the *SacI* ends, after the termini were made flush with DNA polymerase I (Klenow). This change in the ends of the 2.9-kb c-Ha-ras1 fragment was, however, apparently not achieved because when DNAs from two clones (6-2 and 6-9) which hybridized to a Ha-ras probe were analysed, they contained deletions around the inserted sites as shown in the inner circular DNA at the bottom of the figure, instead of the predicted *BamHI* insert shown in the outer circular DNA. There was also a deletion in the 3' sequence of the Ha-MuSV DNA upstream from the LTR. The deletions were 1.4 kb in the 3' sequences of Ha-MuSV DNA, 0.3 kb at the junction between the Ha-MuSV and human c-Ha-ras1 sequences, and 1.8 kb at the junction between pBR322 and human c-Ha-ras1; 1.7 kb of this latter deletion was contained in pBR322 sequences. Despite these deletions, the viral LTR and the region of c-Ha-ras1 homologous to v-Ha-ras were both intact and joined in a single cloned molecule. pBR322 DNA is represented by solid shading, Ha-MuSV DNA by diagonal shading, and human c-Ha-ras1 DNA by cross-hatching. The open rectangles in c-Ha-ras1, which is shown left-to-right in the 5'-3' orientation, represent the exons in the gene.

contain high levels of a p21 which is similar to that specified by the rat gene.

Normal human cells contain low levels of p21

We have recently reported that p21 *ras* is a gene family which consists of at least two prototypic gene types¹⁴: those closely related to *ras* of Ha-MuSV (Ha-ras) and those closely related to *ras* of Kirsten (Ki) MuSV. Ki-MuSV is a rat-derived virus whose v-ras encodes a p21 which is immunologically related to the Harvey p21. Of the four human *ras* genes we have

detected¹³, human c-Ha-ras1 contains three IVS, as does rat c-Ha-ras1, and it is closely related to v-Ha-ras. Two of the other human *ras* genes are more closely related to v-Ki-ras, and one is distantly related to v-Ha-ras.

Because p21 *ras* is a gene family consisting of at least Harvey and Kirsten type genes, it is not clear which of these genes encode the low p21 levels detected in normal cells. The p21 in rodent fibroblasts have recently been found to be predominantly of the Kirsten type¹⁵. This analysis was made possible by the isolation of a panel of eight monoclonal antibodies to v-Ha-ras p21 (ref. 15), the specificities of which have been defined by the viral forms of p21: some recognize Harvey viral p21 but not Kirsten viral p21, whereas others detect both the Harvey and Kirsten forms of p21. In rodents, the type specificity of these monoclonal antibodies apparently also extends to p21 species encoded by Harvey and Kirsten type c-ras genes^{15,16}.

To determine whether human cells contain Harvey type p21, as defined above by the monoclonal antibodies, ³⁵S-methionine-labelled extracts of normal human diploid fibroblasts (WI-38 cells) were immunoprecipitated with a v-Ha-ras p21-specific monoclonal antibody. In contrast to rodent fibroblasts¹⁵, a distinct 21K band was detected with this antibody (Fig. 1). This result suggests that these human cells contain significant levels of Ha-ras p21.

LTR activation of human c-Ha-ras1 transforming activity

The two previously cloned c-onc retroviral homologues, mouse c-mos and rat c-Ha-ras1, which have been shown to possess oncogenic potential, were both inactive by themselves when assayed for their ability to transform NIH 3T3 cells⁷⁻⁹. To demonstrate their capacity to induce morphological transformation, it was necessary to ligate a viral LTR upstream from the

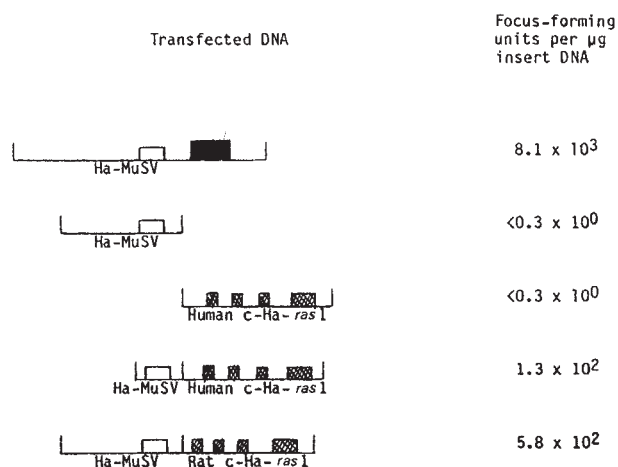
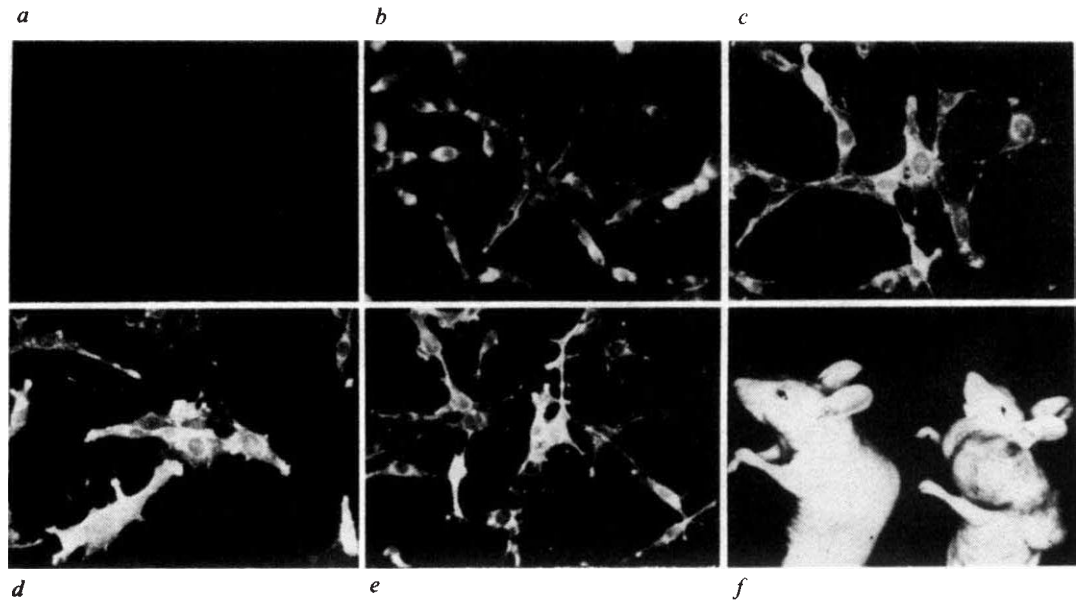


Fig. 3 Transformation induced by the normal Harvey *ras* oncogene. DNAs were precipitated with calcium chloride³⁶ and 0.2 ml was added to 35-mm dishes seeded on the previous day with 2.25×10^5 NIH 3T3 cells. Calf thymus DNA ($25 \mu\text{g ml}^{-1}$) was used as carrier. Each DNA insert shown was cloned in pBR322; 0.1 or 0.2 μ g of insert DNA was used per dish. The DNAs were added without digestion by a restriction endonuclease. The cells were treated as described elsewhere², except that DM50 was not used. Foci were counted 16 days later. The viral LTR is represented by an open rectangle, v-Ha-ras by a solid rectangle, and the exons of c-Ha-ras1 (defined by their homology to v-Ha-ras) by cross-hatched rectangles. Ha-MuSV DNA was genomic clone H-1, which is a 5.4-kb circularly permuted copy of the viral DNA genome^{5,37}; Ha-MuSV LTR DNA was clone P-14, described in Fig. 2 legend; human c-Ha-ras1 DNA contained the 2.9-kb *SacI* fragment also described in Fig. 2 legend. In other experiments, a 6.6-kb *BamHI* fragment of c-Ha-ras1 which contained this 2.9-kb *SacI* fragment also failed to induce foci. Ha-MuSV LTR-human c-Ha-ras1 DNA was clone 6-2 (clone 6-9 gave similar results in other experiments) described in Fig. 2 legend. Ha-MuSV LTR-rat c-Ha-ras1 DNA was clone P-14 into which a 2.3-kb fragment of rat c-Ha-ras1 had been inserted (by M. Haiken, R. Ellis and E.H.C.) at the *BamHI* site. This 2.3-kb fragment of rat c-Ha-ras1 extends from *EcoRI* to *XbaI* in clone LHBE⁹; these sites were converted (by R. Ellis) to *BamHI* by adding oligonucleotide linkers and cloned at the *BamHI* site in pBR322 (this latter clone is called LXBE).

Fig. 4 Detection by immunofluorescent staining of p21 in NIH 3T3 cells transformed by Harvey *v-ras* and *c-ras* genes; tumour formation of human *c-ras* transformants. *a-e*, NIH 3T3 cells and derivatives transformed by Harvey *v-ras* or *c-ras* genes were tested for expression of p21 by indirect immunofluorescent staining with a monoclonal antibody specific for Harvey *ras* p21: *a*, NIH 3T3 cells; *b*, clonal line transformed by Ha-MuSV (viral non-producer Ha821); *c*, clonal line transformed by rat *c-Ha-ras1* ligated to LTR of Ha-MuSV 9; *d*, focus of cells transformed by human *c-Ha-ras1* ligated to LTR of Ha-MuSV (clone 6-2); *e*, focus of cells transformed by human *c-Ha-ras1* ligated to LTR of FeSV. Trypsinized cells were grown on 8-spot printed glass slides (Roboz Surgical) pretreated with 0.1% gelatin, for 18 h at 37 °C in Dulbecco's modified essential medium (DMEM) + 10% fetal calf serum. After washing with phosphate-buffered saline (PBS), the cells were fixed by incubation at 25 °C for 10 min in 3.7% formaldehyde in PBS, followed by incubation in absolute methanol at -10 °C for 4 min. The slides were rinsed briefly in acetone (-10 °C) and air-dried. The fixed cells were incubated at 37 °C for 45 min with monoclonal antibody Y13-238 (ref. 15). The antibody was concentrated and partially purified from culture supernatants of cells grown in serum-free medium, and was used at a final concentration of $\sim 20 \mu\text{g ml}^{-1}$ in PBS with 0.4% (w/v) bovine serum albumin (BSA). After washing with PBS, the cells were incubated at 37 °C for 45 min with rhodamine-labelled antibodies against rat IgG, prepared in goats (Cappel Laboratories). The fluorescent antibody was affinity-purified on a column of rat IgG by Dr Jurgen Wheland, NCI, and was used at a concentration of $125 \mu\text{g ml}^{-1}$ in PBS with 0.4% BSA. The slides were washed extensively with PBS, mounted under coverslips (no. 1) with Elvanol (DuPont), and observed using a Zeiss microscope equipped with epifluorescent optics. The cells were photographed under UV light using a $\times 40$ Planapochromat objective. Photographs were matched for times of exposure and development, except that the development time for *a* was reduced to enhance the low level of background staining; at matched development this panel would be completely black. Controls of normal and transformed cells stained after incubation with normal rat serum or irrelevant monoclonal antibodies are indistinguishable from *a* (data not shown). Final magnification is $\times 105$. *f*, On the left is a nude mouse showing no evidence of a tumour, 3 weeks after subcutaneous inoculation with 2×10^6 NIH 3T3 cells; on the right is a nude mouse having a large tumour at the site where the mouse was inoculated 3 weeks previously with 5×10^5 cells from a focus transformed by clone 6-2 (human *c-Ha-ras1* ligated to the LTR of Ha-MuSV). In this experiment none of five mice inoculated with 2×10^6 NIH 3T3 cells developed tumours after 10 weeks, whereas all of five mice inoculated with cells from the lines shown in *b-e* developed palpable tumours within 2 weeks.



5' end of the gene. Similarly, attempts to induce cellular transformation with the human *c-ras* genes without an LTR have been unsuccessful. In our initial attempts to transform cells with the *c-ras* genes, we have focused on human *c-Ha-ras1*, which preliminary molecular hybridization data have suggested is transcriptionally active in normal human fibroblasts (E.H.C. *et al.*, unpublished data).

We therefore constructed a chimaeric plasmid containing the LTR of Ha-MuSV upstream from human *c-Ha-ras1* (see Fig. 2). This chimaeric DNA induced focal transformation of NIH 3T3 cells in all of four separate experiments. While the foci induced by Ha-MuSV DNA were usually first detected about seven days after addition of the DNA to the cells, the foci induced by human *c-Ha-ras1* were generally detected about nine days after addition of the DNA; this short delay has also been observed for rat *c-Ha-ras1*. The results of a typical experiment are shown in Fig. 3. The number of foci induced by a chimaeric plasmid containing an LTR upstream from rat *c-Ha-ras1* was an order of magnitude lower than that induced by a circularly permuted full-length Ha-MuSV DNA genome. The clone containing human *c-Ha-ras1* induced only about one-fifth as many foci as did the rat *c-Ha-ras1* clone. The higher efficiency of transformation by the chimaeric plasmid containing rat *c-Ha-ras1* may be due to the fact that it carries almost 2 kilobases (kb) more non-*ras* Ha-MuSV sequences upstream from the LTR than were present in the chimaeric plasmid with human *c-Ha-ras1*; deletion of these sequences has previously been associated with a lower efficiency of transformation by Ha-MuSV DNA^{5,17}. In further experiments, three independent foci were also obtained in the NIH 3T3 cells when the human *c-Ha-ras1* fragment (a 2.9-kb *SacI* fragment¹³) was ligated without cloning to a 1.4-kb *SacI* fragment which contained the cloned LTR of the Gardner-Arnstein strain of feline sarcoma virus (FeSV)¹⁸.

In addition to being phenotypically transformed, the cells transformed by human or rat *c-Ha-ras1* were also tumorigenic for nude mice: palpable tumours developed in less than two weeks in mice inoculated with 5×10^5 transformed cells, whereas no tumours were evident in the 10 weeks following inoculation with 2×10^6 control cells (see Fig. 4f).

Human gene transformants contain the LTR and human *c-Ha-ras1*

To determine the presence of the cloned DNA in the transformed NIH 3T3 cells, genomic DNA from transformants was analysed for the presence of LTR and human *c-Ha-ras1* sequences. Genomic DNAs were digested with restriction endonuclease, and fragments were fractionated by agarose gel electrophoresis, transferred to nitrocellulose paper, and hybridized with ³²P-labelled LTR and human *c-Ha-ras1* probes (Fig. 5). Hybridization to the *ras* probe of *EcoRI*-digested genomic DNA from a transformant yielded two major new fragments of 7.6 and 5.8 kb (Fig. 5a, lane 3)—this indicated that the transformant contained additional *ras* sequences. These new fragments were presumably human *c-Ha-ras1*, since at the exposure in the autoradiogram shown in Fig. 5, the probe detected the 25-kb *EcoRI* fragment in human genomic DNA previously identified as containing *c-Ha-ras1* (ref. 13; Fig. 5a, lane 1) and did not reveal endogenous mouse *ras* fragments in the NIH 3T3 cells (Fig. 5a, lane 2). The LTR was presumptively identified as being linked to both of these fragments, as hybridization of *EcoRI*-digested DNA from the transformant to the LTR probe detected, in addition to multiple copies of endogenous LTR fragments, two novel fragments which comigrated with the two fragments identified using the *ras* probe (Fig. 5b). The 5.8-kb *EcoRI* fragment is similar in size to the input cloned DNA, suggesting that some of the copies might still be intact circular episomes or integrated in tandem.

extend this finding to a human *c-onc*, a species from which a transforming retrovirus has not yet been isolated. These results suggest that such transformation may be a general feature of *c-Ha-ras1* genes. It should also be stressed that these rodent and human genes have been cloned from normal cellular DNA, and they have not by themselves induced focal transformation of NIH 3T3 cells.

Given the tumorigenic properties of these activated normal genes, it seems reasonable to speculate that other cellular oncogenes may possess analogous tumorigenic potential, and that normal transcriptional control mechanisms prevent these genes from being continuously expressed at high levels. These and other examples of LTR-mediated activation of a cellular oncogene^{8,9,24-26} suggest that a constitutive change in the transcriptional control of an oncogene could represent an important mechanism in the pathogenesis of some cancers. Such changes in oncogene expression might be induced either by viral or other mechanisms, such as somatic mutation or recombination. In this regard, it has recently been reported that, in contrast to the very low transforming potential of genomic DNA from

normal cells, genomic DNA from some human malignancies is able by itself to induce oncogenic transformation of NIH 3T3 cells²⁷⁻²⁹. These transforming sequences from a bladder carcinoma have recently been molecularly cloned and have retained their oncogenicity³⁰⁻³². As reported in the accompanying paper³³, this cloned gene has now been identified as human *c-Ha-ras1*. Similar observations have also been made by others^{32,34}. Because the normal *c-Ha-ras1* gene and the human tumour *c-Ha-ras1* gene are now available, it should be possible to localize the alterations in the normal gene that are responsible for the dramatic change in biological activity of the gene isolated from the bladder tumour. This analysis may help to elucidate the molecular basis of neoplastic transformation in a human cancer.

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LETTERS TO NATURE

Lithium abundance at the formation of the Galaxy

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The observed abundance of lithium in population I stars (young stars)¹ seems to have been stable during the past 5×10^9 yr (ref. 2). This stability has been used previously^{1,3} to estimate the abundance of lithium at the time of formation of the Galaxy and at pre-galactic times^{1,3}. But because the basis for this estimate was not completely satisfactory, we have made new observations, aiming at a better determination of the lithium abundance at the time of formation of the Galaxy. The newly observed stars are extreme population II dwarfs (very old, very metal-poor stars). Their lithium abundance turned out to be significantly lower than the abundance in population I stars. If attributed to the big bang, this lithium abundance suggests a rather low baryonic density of the Universe, which in turn favours, under some assumptions, an open Universe³. Finally, we suggest that the good agreement between the abundance of ⁷Li and the deuterium ²H abundance³ supports the standard model of the big bang.

The new Canadian, French, Hawaiian (CHF) 3.6-m telescope⁴ with its large collecting area, excellent seeing conditions and

the sensitivity of its Reticon receiver has made it possible to observe the lithium resonance line⁵ in dwarf stars of the extreme population II, often called halo dwarfs (typical representative of the halo of the Galaxy). These stars are very old and their metal deficiency reflects the lack of metals at the time of their formation. They are faint and the lithium line is narrow and faint in such stars, so that its measurement requires high resolution spectra with a high signal-to-noise ratio. Good spectra were obtained for a few of these stars and lines as faint as 3 mÅ can be detected. A comparatively strong lithium line (23-50 mÅ) was observed^{5,6} in all except the coolest star. This absence of lithium is easily understood when it is recalled that the convective zone is very deep in cool stars: the lithium is then driven down into very hot layers, where it is completely destroyed by proton fusion⁷.

From the measured wavelength of the observed lithium line it seems that the absorbing species is essentially the ⁷Li isotope.

A classical spectrum analysis using model atmospheres has been carried out to determine the abundance of lithium for each star. The high quality of the spectra means that the error on the equivalent widths is negligible. The random error in determination of the lithium abundance (n_{Li}) comes essentially from the lack of precision about the effective temperature (T_{eff}) of the stars. For this type of stars it is generally admitted that $\Delta \log T_{\text{eff}} = 0.01$. If $\log T_{\text{eff}}$ is increased by 0.01, then $\log n_{\text{Li}}$ will be increased by about 0.2 (Fig. 1).

Clearly, the present sample of Li abundances in population II dwarfs is very small; however, the abundance of lithium is

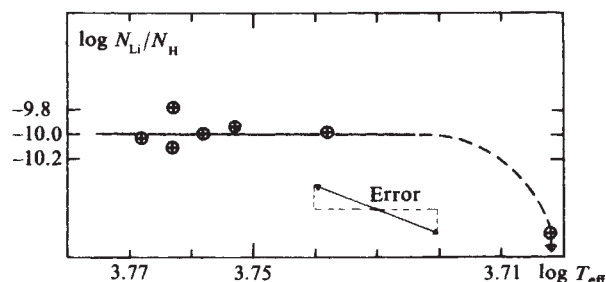


Fig. 1 Lithium abundance plotted against effective temperature (logarithmic scale) for very old metal-poor stars. The lithium abundance is remarkably constant except for the coolest star: here, deep convective movements carry the lithium into very hot layers where it is completely destroyed.

remarkably constant in all the observed halo dwarfs but the coolest one (Fig. 1). This constancy versus the effective temperature T_{eff} of the stars is also a measure of constancy versus the mass of these essentially unevolved stars. If confirmed by more observations, this suggests that lithium is not destroyed by convection in solar type stars of population II, at variance with what is assumed⁸ in population I stars. Taking advantage of this constancy, it is easy to show that the lithium abundance (relative to hydrogen) in the atmospheres of old halo dwarfs is $N_{\text{Li}}/N_{\text{H}} = 10^{-10}$ —about 10 times less than the population I abundance¹.

This lithium is not expected to come from the inside of the star: nucleosynthesis of lithium has been predicted only for stars in a much more advanced stage of evolution². Nor is the ^7Li expected to come from outside: most of these are stars whose velocity, relative to population I interstellar clouds, is high, so that the accretion process is inefficient^{10,11}.

The lithium observed in the halo dwarfs has its origin in the lithium existing in the material which formed these stars. The abundance of lithium found in the atmosphere of the star should not be very different from the abundance in this material. Admittedly, several causes of depletion of lithium may exist: some lithium may be destroyed during the contraction of the protostellar cloud^{7,12} (pre-main sequence phase); microscopic and/or turbulent diffusion (acting during the very long life in the main sequence of these very old stars) could deplete the lithium¹³; the stellar mass-loss acting over a long time could also cause depletion (H. Reeves, personal communication). All these effects are not yet well known for halo stars; moreover, at first sight it could be conjectured that they are mass (and/or temperature) dependent; this was confirmed by other observations, and the constancy of the lithium abundance suggests that these processes are not very efficient. Keeping in mind the causes of uncertainty, let us assume that $N_{\text{Li}} = 10^{-10} N_{\text{H}}$ is the abundance of the material which formed the halo dwarfs. This abundance cannot be significantly different from the abundance of the pre-galactic material: admittedly, the atmosphere of the halo dwarfs includes some material processed in supernovae; this material, when astrated, was on the one hand deprived of its lithium, and on the other hand strongly enriched in metals. As the halo dwarfs are metal poor, only a very small fraction of the astrated material can have been mixed in the pre-galactic material; the lithium abundance seems constant within rather wide limits of metal abundance (1/20–1/200 of the solar metal abundance). It seems therefore that we can take the lithium abundance of the atmospheres of the halo dwarfs as the abundance of the pre-galactic material.

Evaluated by mass, this pre-galactic lithium abundance is $X_7 = 5.2 \times 10^{-10}$ (assuming a helium abundance $Y = 0.25$ by mass). If attributed to the big bang, this abundance leads¹⁴, according to the standard theory, to a rather low baryonic density of the Universe (Fig. 2) lower than the density previously derived by Boesgaard¹ and by Austin and King³. The density found remains, however, in agreement with that derived from

the abundance of the deuterium³, within the estimated errors. For an easier comparison with the work of Austin and King, the Hubble constant retained was $H_0 = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

The error of the primordial lithium abundance determination may be estimated as follows.

(1) The classical abundance analysis, using models of atmospheres, with a few simplifying assumptions (such as local thermodynamical equilibrium), may induce an error as high as a factor of 2 (about $10^{0.3}$ or 0.3 dex). However, the systematic part of this error is about the same for populations I and II, so that it cancels out, at least partly, when comparing relative abundance in populations I and II. When using several stars, the random part of the error decreases accordingly, even when adopting the parameters T_{eff} and g as they appear in the literature (without homogenization).

(2) There is some uncertainty about the possible depletion of lithium, owing to the possible proto-stellar destruction and/or depletion due to diffusion, and/or lithium depletion due to stellar wind. This uncertainty is difficult to estimate. We can adopt, as an upper limit, a factor of ~ 3 (0.5 dex), because Duncan⁸ and Bodenheimer¹² predict only a factor of 2 for proto-stellar destruction for solar type stars.

Thus taking account of the cumulated errors, the final uncertainty about the difference between the lithium abundance in populations I and II is probably less than a factor of 6 (0.8 dex); so that we have to accept that the abundance of lithium is probably lower in population II than in population I.

It seems that the evolution of the Galaxy has not significantly decreased the abundance of the primordial lithium²; as lithium is destroyed inside stars and stellar envelopes, a lithium-producing process must be found. Spallation is only partially responsible for this production¹⁵; a promising possibility is production in novae¹⁶.

Even considering the uncertainties involved in the interpretation of the lithium abundance in halo dwarfs, our work provides a clear demonstration that the Universe cannot be closed by

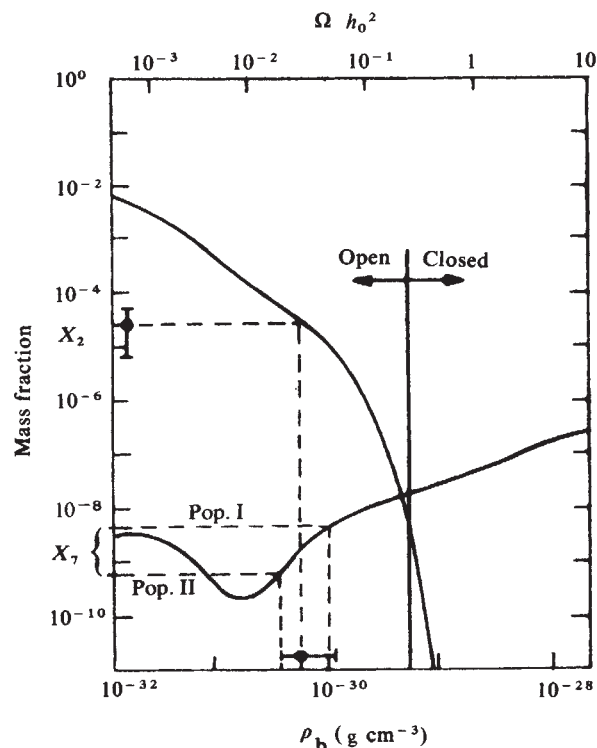


Fig. 2 The new lithium abundance X_7 found in this work leads to a rather low baryonic density, in agreement (within determination errors) with that derived from deuterium abundance. The values of the Hubble constant is the same as in ref. 3. If the cosmological constant Λ is assumed to be zero, this leads, more clearly than in previous work, to the conclusion that the baryonic density is not large enough to close the Universe.

nucleons, when assuming the validity of the standard model¹⁴ of the big bang and assuming a zero value for the cosmological constant ($\Lambda = 0$). This result remains unchanged when compared with the model of Yang *et al.*¹⁷, which has three pairs of neutrinos ($\Omega_N h_0^2 = 2 \times 10^{-2}$). More observations, a better theory of protostellar and stellar convection, a better theory of diffusion and a better theory of stellar wind are needed to obtain a more accurate value of the primordial lithium abundance. However, even within the present uncertainty, some agreement exists between the baryonic density predicted from lithium and deuterium abundances, and when confirmed, this agreement will support the validity of the standard model of the big bang.

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Five-minute oscillations as a subsurface probe of sunspot structure

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Observations are reported here which show that the 5-min oscillations in a sunspot umbra actually split into several individual modes of different period. We interpret these modes of oscillation as the response of the sunspot to forcing by the 5-min p-modes in the surrounding quiet atmosphere. Also, we show how detailed observations of the multiple 5-min modes in a sunspot may be used as a probe of the structure of a sunspot beneath the solar surface.

Oscillatory velocity fields in sunspots may be divided into two categories. The first category consists of the umbral oscillations (period 145–190 s) and the running penumbral waves (period 180–250 s). Observations suggest that these waves are resonant modes (see refs 1, 2), and theoretical work^{3–7} has identified these waves with resonant magneto-atmospheric wave modes of the sunspot atmosphere itself. These waves, which are presumably excited by subsurface oscillatory convection, offer indirect evidence of the existence of overstable convective modes in the subphotosphere.

The other category of oscillations in sunspots consists of oscillations with periods around 5 min, the dominant period of oscillations in the quiet photosphere. Recent observations^{8–10} have overcome the problems of stray light from the surrounding

photosphere and the lack of an absolute wavelength reference and have confirmed the existence of 5-min oscillations in sunspot umbras, at power levels well below that in the quiet photosphere. As there are no resonant modes of sunspot oscillations in the 5-min range, we have suggested¹¹ that these 5-min oscillations represent the passive response of the sunspot to forcing by the 5-min p-mode oscillations in the surrounding gas. It is now well established that the 5-min oscillations in the quiet photosphere actually consist of several modes of oscillation, in the form of acoustic waves trapped in the upper convection zone (p-modes)¹². These p-modes include a wide range of horizontal wavelengths with a moderate variation of frequency. It is expected that the geometry of the sunspot and the nature of the coupling between the acoustic waves and the sunspot flux tube will act as a filter, with only certain p-modes forcing the sunspot with observable amplitude.

The present observations were made as part of a large programme to study dynamical phenomena in sunspots, using the vacuum tower telescope and Echelle spectrograph at Sacramento Peak Observatory. Here we show only the results of two high-quality time series of velocity oscillations in the umbral photosphere measured in the spectral line Fe I $\lambda 6,302.5$. The velocity was measured from photographic spectra using the Doppler shift of the magnetic V profile of the line, thus assuring that the origin of the signal is from within the sunspot and not due to scattered light. Also, a nearby telluric line of

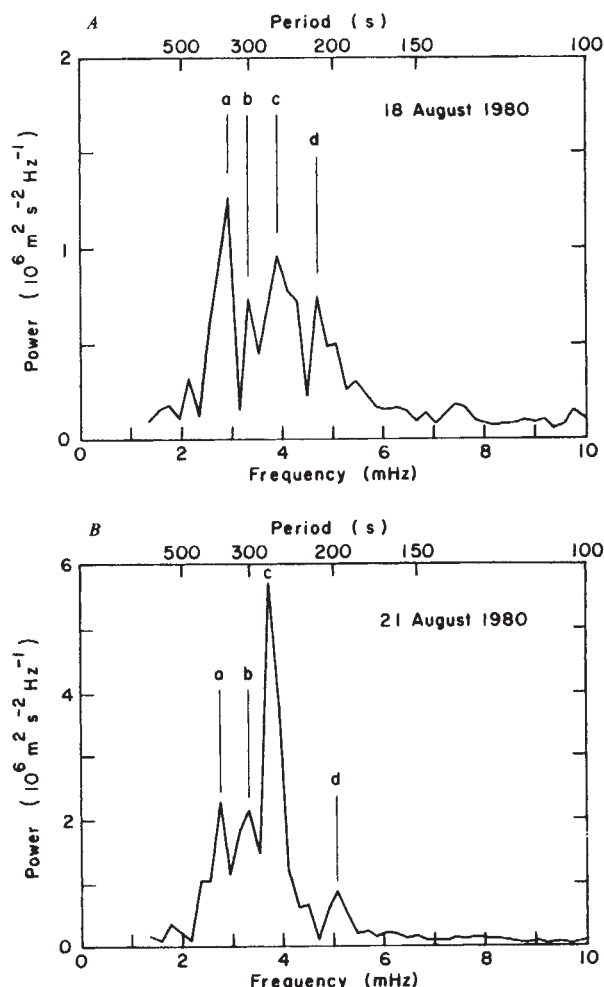


Fig. 1 The mean power spectrum of line-of-sight motions in the umbral photosphere for two runs: 18 August 1980 (A) and 21 August 1980 (B). The peaks have the following periods: A: a, 353 ± 6 s; b, 301 ± 4 s; c, 263 ± 3 s; d, 213 ± 5 s; B: a, 366 ± 6 s; b, 301 ± 4 s; c, 269 ± 4 s; d, 197 ± 4 s. Peaks a, b and c are interpreted as representing the response of the sunspot to individual p-mode oscillations in the surrounding atmosphere.

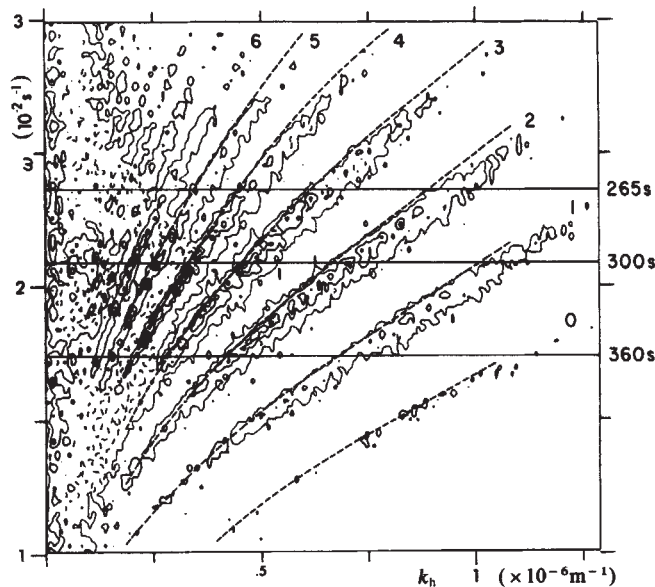


Fig. 2 Highly refined diagnostic diagram for the solar p-modes, due to Deubner *et al.*¹⁴, showing contours of power on the frequency–horizontal wavenumber plane. Lines corresponding to the nominal periods of umbral oscillation near 5 min (360 s, 300 s and 265 s) are drawn to determine their points of intersection with p-modes of different radial mode number n (also shown).

O₂ was used as a wavelength reference, thus avoiding the dangers inherent in using a photospheric line as a velocity reference.

Figure 1 shows the mean power spectrum of the velocity field for the portion of the umbra covered by the entrance slit, for each of two runs (18 and 21 August 1980). Each power spectrum has three conspicuous peaks clustered around a 5-min period. Given the frequency resolution of our observations, the periods of these three peaks for both runs are consistent with nominal values of 265 s, 300 s and 360 s. Another conspicuous peak near 200 s in both runs is more likely to be associated with a resonant mode of the sunspot itself. The greater power in the peaks on 21 August, when the sunspot crossed the central meridian of the Sun, indicates that the velocity is predominantly vertical in the photosphere. The total power at periods near 5 min implies an r.m.s. vertical velocity of $\sim 50 \text{ m s}^{-1}$ in the umbra, at least a factor of five smaller than for the 5-min oscillation in the quiet photosphere.

Because of the limited temporal and spatial extent of the data (70-min time series with a 20-s sampling interval, over about $13 \times 10^6 \text{ m}$ in the umbra) there is statistical noise in the power spectrum. The noise level may be estimated in three ways: from the high-frequency limit of the power spectrum, from the statistics of the distribution of individual power spectra that are used to form the mean power spectrum for the entire umbra, and from the estimated number of degrees of freedom (~ 20) using Edmond's tables¹³. All three of these methods indicate that the 50% confidence level for each peak corresponds to roughly 25% of the peak amplitude. Any peak that is four times the noise level is marginally significant (50% confidence level), with greater confidence for higher peaks. Thus, in Fig. 1, the peaks near 360 s, 300 s, 265 s, and 200 s are all significant at well above the 50% confidence level. Note that the noise level is about the same on the two days while the signal increases in power on 21 August as the line-of-sight velocity becomes more nearly vertical.

We interpret the three periods of oscillation around 5 min (265 s, 300 s, and 360 s) as the response of the sunspot to three distinct modes of 5-min oscillation in the surrounding photosphere. We pursue this by plotting these three periods, in Fig. 2, on a highly refined k - ω (wavenumber–frequency) diagram for the photospheric oscillations (due to Deubner *et al.*¹⁴). Note that the three umbral periods cross all of the p-mode ridges in

the k - ω diagram. We may identify the three umbral periods as the response of the sunspot to three consecutive p-modes, but there is ambiguity in choosing the correct set of p-modes. Each set of three mode crossings corresponds to a set of three horizontal wavenumbers and three corresponding horizontal wavelengths of p-mode oscillation. One might expect some relation between the diameter of the sunspot umbra and the wavelength of peak response to the surrounding p-mode excitation. The observed sunspot umbra is irregular in shape and has an area of $\sim 170 \times 10^{12} \text{ m}^2$, giving an effective diameter (diameter of a circle of the same area) of $14.7 \times 10^6 \text{ m}$. (This estimate is subject to the uncertainty in locating the umbral–penumbral boundary by photographic methods). This surface umbral diameter falls within the range of possible wavelengths. However, because the diameter of the sunspot should decrease with depth (due to increasing pressure in the surrounding convection zone), the surface diameter is probably not an appropriate value for comparison.

We suggest that observations of the multi-mode structure of 5-min oscillations in sunspots may be used as a probe of sunspot structure below the solar surface. The various p-modes have eigenfunctions with different depth dependence, with higher modes extending deeper into the convection zone, so that each mode 'probes' a different range of depths. This fact has already enabled the p-modes to be used as a probe of radial variations in the solar rotation rate in the convection zone^{14–16}. Here we suggest a similar technique to probe the structure of a sunspot below the surface by measuring the response of the sunspot to the various p-modes. This method calls for accurate observations of the period and spatial structure of the various 5-min modes in the sunspot as well as theoretical work on the interaction between the p-modes and the sunspot flux tube.

An illustration of such a depth probe can be given on the basis of the present observations and a simple theoretical argument. Space–time plots of the umbral velocity field show a characteristic 'herringbone' structure with phase varying across

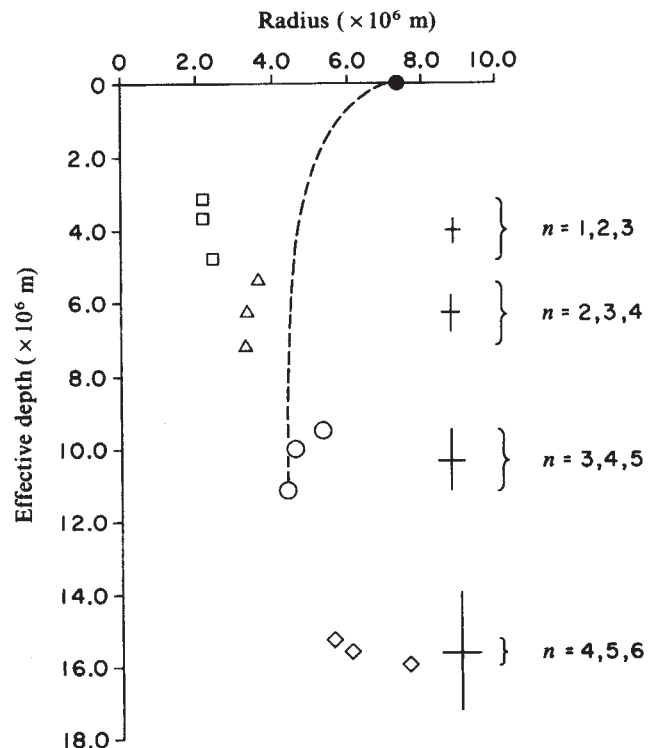


Fig. 3 Four possible choices of sunspot radius versus effective depth as determined by four different sets of three consecutive p-mode crossings in Fig. 2. The crosses show the estimated error for each set (see text). ●, The observed surface radius of the sunspot. O, The most likely set of mode crossings. The dashed line gives the variation of sunspot radius with depth for Deinzer's¹⁷ sunspot model, and is shown for comparison only.

the umbra. At any instant, diametrically opposite points at the edge of the umbra tend to be 180° out of phase and the centre of the umbra appears to be a node in the velocity field. This suggests that the peak response is occurring at a wavelength equal to roughly twice the umbral diameter. We will assume that the peak response is at a wavelength equal to exactly twice the local diameter and proceed. As discussed above, the various sets of three consecutive mode crossings in Fig. 2 predict alternative sets of three horizontal wavelengths and hence sets of three values of sunspot diameter. Each diameter corresponds to a different p-mode (different value of n) which probes a different effective depth. Values of the effective depth for the p-mode eigenfunctions have been calculated by Ulrich *et al.*¹⁶. The effective depth varies with horizontal wavenumber as well as radial mode number. For any set of mode crossings in Fig. 2 we can assign an effective depth to each of the three values of sunspot diameter and thus obtain a plot of sunspot radius versus depth, assuming a circularly symmetric sunspot flux tube.

The results of these calculations are combined in Fig. 3, where sunspot radius is plotted as a function of depth for each of the possible sets of mode crossings. The limited resolution

in frequency (due to finite time series) and the spread of power in the ridges on the k - ω diagram give the uncertainty in calculated sunspot radius, and the uncertainty in k and ω gives the uncertainty in effective depth. For any given set of points in Fig. 3 the uncertainties mask the depth variation of sunspot radius, making each set of three points equivalent to a single piece of information about the subsurface sunspot geometry. The individual sets of three points in Fig. 3, corresponding to sets of three consecutive mode crossings, are distinct from each other, allowing a selection of the most likely set based on theoretical arguments about the expected depth variation of sunspot radius. For example, the theoretical model of Deinzer¹⁷ is plotted in Fig. 3 for comparison and passes through the set of points corresponding to p-modes $n = 3, 4$ and 5 . We use this only as an illustration of the method and not as a specific prediction for this sunspot. However, for the range of wavenumbers under consideration, modes $n = 3, 4$ and 5 have the most power in their ridges; they might be chosen on this basis as well.

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Direct gravimetric detection of magma movements at Mount Etna

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Mount Etna is Europe's most active volcano, erupting some 2×10^{10} kg of new material each year¹. Its eruptions generally have two distinct forms: continuous explosive activity near the summit with low rates of lava effusion, or massive, and often quieter, lava effusion from its flanks along regional tectonic fissures. The question of where magma is stored before eruption is not yet resolved. Here I present results from high-precision gravity surveys covering two recent eruptions. Gravity changes after the major flank eruption of March 1981 indicate draining of magma from a dyke 14 km long, at an elevation of 0–1 km and striking in a direction close to that of the eruptive fissure. Changes from the explosive sub-terminal eruptions of September 1980 do not indicate draining from any near-surface reservoir. It is proposed that short-term magma storage can occur close to the surface before a flank eruption, but that summit eruptive activity is consistent with a source at greater depth.

Recent research on Mount Etna has shown that it does not conform to the classic inflation–deflation model² which can be applied successfully to many other volcanoes, notably Kilauea^{3,4}. Precise levelling and dry tilt studies show no gross systematic deformation, but only local anomalies⁵, and horizontal deformation observed in the summit region is inconsistent with a simple sub-surface reservoir⁶. Furthermore, petrological studies on historical lava flows indicate little of the chemical differentiation which would be expected if substantial high-level magma storage were involved^{7,8}, and seismic investigations indicate a large volume of partial melt at a depth of 16–24 km beneath the volcano⁹. It has been concluded that magma rises directly from great depth to the surface without long-term storage in a magma chamber^{10–12}. It is accepted, however, that

high-level storage must take place in the short term, because all lavas show extensive crystallization of plagioclase, indicating residence at moderate to low pressures¹², and some flank effusions are only mildly explosive, indicating degassing before eruption. The lavas of 1910, 1928, 1950–51 and 1971 also show a limited degree of magmatic evolution due to differentiation^{13–14}. This implies high-level storage on a time scale sufficient to give rise to crystallization and degassing, but insufficient to result in strong differentiation. To address the problem of where magma resides before eruption, a high precision gravity network was established on Mount Etna in September–October 1979 (ref. 15) with the aim of detecting small secular changes in the force of gravity as a result of intrusion or draining of matter.

Ninety-seven gravity stations have been set up, of which 48 are used in the surveys under discussion (Fig. 1a). They are sited on stable massive lava outcrops or permanent architecture. Station relocation is good to ± 1 cm, and vertical reference to ± 1 mm. Instrument orientation is constant at each station. Measurements are carried out with LaCoste and Romberg gravity meter G90, fitted with electrical readout. Tidal corrections are made using a harmonic tidal programme based on Cartwright and Tayler^{16,17} and using gravimetric factors and anomalous phases determined for Mount Etna by Mohamed¹⁸. Tides and drift are removed using a least-squares optimization programme. Gravity values are referred daily to a base station at the Astrophysical Observatory at Serra la Nave and the network as a whole is referred to a datum based on this and calibration stations numbers 59, 61 and 64 of the Italian Geodetic Commission. The typical standard deviation of a single gravity tie with meter G90 is found to be $9 \mu\text{Gal}$, and the errors are normally distributed. Each station tie is performed if possible eight times on independent occasions, and it is estimated that calibration variations, datum inaccuracies and water-table effects contribute a further nominal error of $5 \mu\text{Gal}$. The resulting error for a gravity change is typically 8–15 μGal , depending on the number and quality of the ties made. In the interval September 1979–August 1980 gravity changes in the range -36 to $+48 \mu\text{Gal}$ were observed. A χ^2 test gives a probability of $<1\%$ that they are due to chance.

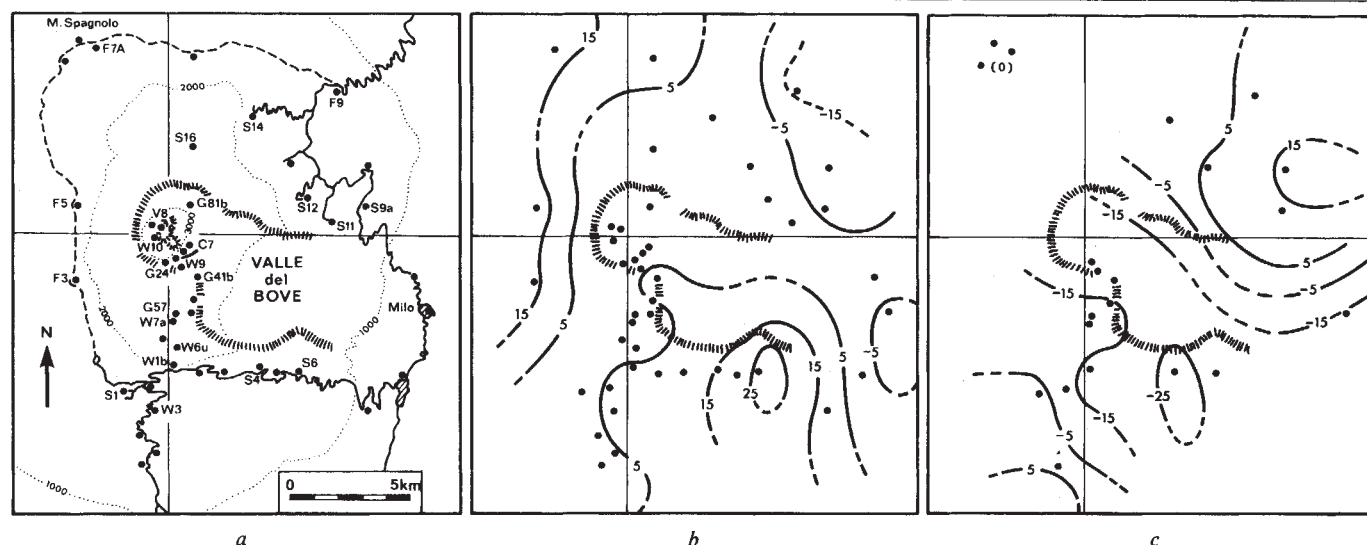


Fig. 1 *a*, Map of Mount Etna: ●, gravity stations; hatching, caldera rims. Topographic contours are in intervals of 1,000 m. *b*, Smoothed contours to show broad features of gravity changes between August and September 1980. Contours are plotted by computer, using a routine in which the gravity change value at any point is calculated as a weighted average of all measurements within a circular window around it. Measurements are weighted inversely to variance and the square of distance from any station, and are smoothed using a filter of cutoff wavelength 0.750 km. A window of radius 5 km is used. Contours are at intervals of 10 μGal , and are dashed where information is poor. Details of the contours should not be taken seriously. *c*, Smoothed contours to show broad features of gravity changes September 1980–March 1981.

A change in the measured force of gravity can be due to two effects. First, an uplift of terrain leads to a reduction in gravity according to the inverse square law. This reduction is $\sim 0.22 \text{ mGal m}^{-1}$ and can be used for the detection of vertical elevation changes¹⁹. Second, it can be due to sub-surface density changes or movements of matter. To distinguish between these effects 12 of the gravity stations are common to precise levelling stations of Murray and Guest⁵. They were measured simultaneously with the levelling traverse in September 1979 and July 1980. During this period gravity changes of between -36 and $13 \mu\text{Gal}$ were observed at stations for which the elevation changes were respectively -0.6 cm and 0.4 cm (J. B. Murray, personal communication). If the gravity changes were due to elevation changes the corresponding elevation changes would be $+16.4 \text{ cm}$ and -5.9 cm . The observed changes are not only too small by an order of magnitude, but they are of opposite sense. This result, and the fact that some 35 dry tilt stations covering the volcano show no pattern of inflation or deflation⁵, is a justification for interpreting the gravity changes as due to sub-surface accumulation of magma without corresponding ground deformation.

Such accumulation, whether the magma reservoir is in the form of a dyke system or a cylindrical conduit, can occur in several ways. If the reservoir is connected to an open surface, degassing to the atmosphere occurs, leaving the magma more dense. If the system is closed, it can accommodate the introduction of matter through bubble compression²⁰ or through bulk compression²¹. Whether the system is open or closed, magma may be introduced at the expense of trapped gases, which are either compressed, absorbed or simply expelled. Mount Etna can be considered, on the basis of tectonic considerations and observation of surface fissure trends, as a system of vertical radial dykes extending along major regional faults²². It is proposed here that magma is rising passively into vertical fissures in which the gas pressure and magma level are variable. The magma is not intruding actively through consolidated rock and causing great elastic strain; it is effectively filling a void. Eruption can then occur as a result of tectonically controlled block movements and swelling of magma as pressure is released²². If limited surface deformation does occur, as was observed for 2 yr before the 1978 eruption^{5,12}, this is due to stresses associated with the changing level of magma in the fissure, but the strain involved is not a reflection of swelling equal in volume to the magma newly introduced. A similar, though less extreme,

discrepancy has been observed between surface deformation and apparent subsurface volume change on Kilauea²³.

The observed gravity changes on Mount Etna in the period 1979–80 are readily interpretable in terms either of known summit morphology changes or of density changes in historical fissure zones²⁴. Dykes involved in the modelling are typically some 500 m below the surface, of vertical extent $\sim 1,000 \text{ m}$ and of width 1–5 m. However, the parameters are poorly constrained, as the amount of matter gained or lost is not known independently.

In the case of an observed eruption accurate estimates can be made of the amount of material lost from the volcano. Known gravity changes then enable tight constraints to be placed on regions from which it could have come. Consider the

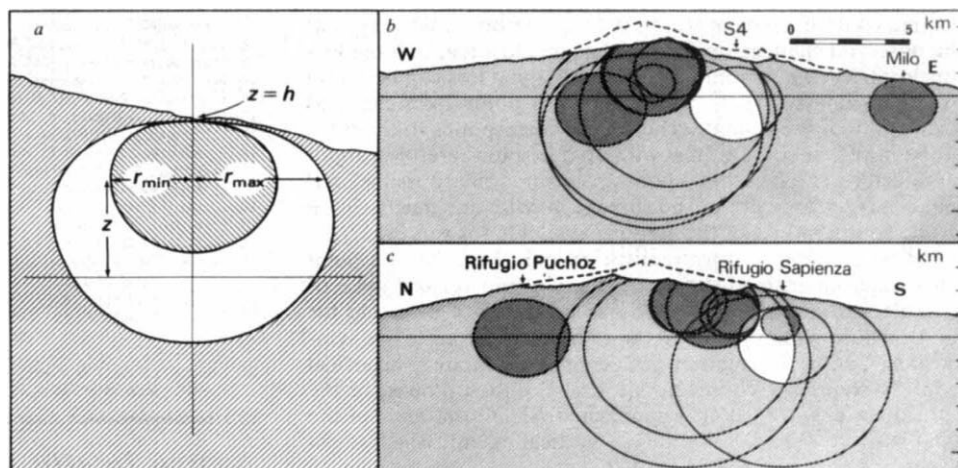
Table 1 Measured gravity changes (μGal) for intervals August–September 1980 and September 1980–March 1981, with associated errors

Location	Station	Gravity change August– September 1980	Error	Gravity change September 1980– March 1981	Error
Rifugio Sapienza	W1b	7	7	−19	7
	W6u	−1	8	—	—
Belvedere Torre del Filosofo Vulcarolo	G57*	−6	8	−11	8
	G41b*	10	8	−22	8
	W9*	8	7	−20	8
	C7*	0	16	−13 (−25)	17
Summit car park	G24*	3	8	—	—
	W10*	−9	8	—	—
	V8	−9	9	—	—
	G81b*	−7	10	—	—
Mount Pizzillo	S16	−3	10	—	—
Rifugio Puchoz	S14	6	8	−3	8
Rifugio Citelli	S12	−8	12	—	—
	S11	2	8	—	—
	S9a	−11	12	13	12
	S6	35	11	−9	13
Serra la Nave	S4	19	9	−33	8
	W3	14	10	—	—
	S1	−3	7	4	8
	F3*	29	10	—	—
Mount Spagnolo da Filippo	F5*	26	8	—	—
	F7a*	17*	13	−4 (5)	8
	F9*	−22	13	8	11

Only those stations measured with accuracy $12 \mu\text{Gal}$ or better are included. The two results in parentheses are corrected for elevation (see text).

* Elevation control exists.

Fig. 2 *a*, Forbidden regions for point mass magma source. Hatching from top left to bottom right indicates a region too close to a station; hatching from bottom left to top right indicates a region too far from one. Sections are shown: *b*, W-E, 4 km south of the summit and *c*, N-S, 2 km east of the summit. The summit profile is shown as a dashed line. The blank region is permissible.



eruption of March 1981. The eruption was only mildly explosive, and followed a series of smaller but highly explosive eruptions during the winter from the North-East Crater²⁵. On 17 March 1981, a fissure opened in the north-west flank of the volcano at elevation 2,250 m and gave rise to strong lava fountaining and massive lava effusion. During the next 6 days the fissure system lengthened to 7.5 km in a north-northwest direction and gave rise to an areal coverage of 4.72 km² of lava, which reached the River Alcantara at 800 m elevation and threatened the town of Randazzo (Fig. 3*d*). The total volume of lava is estimated at 18.6×10^6 m³, a mass of about 4.7×10^{10} kg (estimate by J. B. Murray, from mapping by J. E. Guest, C. R. J. Kilburn, R. M. C. Lopes, J. B. Murray, H. Pinkerton and S. Scott).

A gravity survey was made immediately after the eruption and compared with readings taken in September 1980. The results are corrected for the attraction of new lavas lying on the surface. All those results with error bars better than 12 μ Gal are tabulated in Table 1 with associated errors, and are plotted as smoothed contours in Fig. 1*b*. They show a substantial decrease at all stations high on the southern flank. Towards the region of Rifugio Citelli observed changes were positive or insignificant and at a station 0.5 km from the rift itself there was no significant change. Snow cover prevented measurement of stations in the northern upper reaches and in the Valle del Bove. Snow cover also prevented elevation change control. However, precise levelling surveys have been carried out in August 1981. Two gravity stations showed unusually high elevation changes: station C7, close to the central craters, showed -4 cm (J. B. Murray, personal communication), and station F7a, 0.5 km from the rift, showed +3 cm (M. Grimaldi, personal communication). None of the other stations discussed here showed changes of more than 1.5 cm. As these measurements were made over the interval July/August 1980–August 1981 they are not strictly comparable with gravity changes measured between September 1980 and March 1981, and have not therefore been used to apply an elevation correction to the gravity results. The effect of applying such a correction to results at stations C7 and F7a is included in Table 1, and does not materially alter the picture.

Let us assume that the changes are due to the eruption, and begin with the hypothesis that the erupted magma came from one localized region within the volcano. The magma is equivalent to a sphere of radius 160 m and can be treated as a point mass for depths of the order of 1 km. Now, at station G41b, for example, the measured gravity change was $-22 \mu\text{Gal} \pm 8$. At the 95% confidence level the gravity change therefore lies between -38 and $-6 \mu\text{Gal}$. This range of possible values defines 'forbidden' regions from which the magma could not have come. If a station elevation is h above sea level and the total mass erupted is M , then magma loss is permitted only from a region of radius r from a vertical axis beneath the station

(Fig. 2*d*), where r is a function of elevation z , and satisfies the inequality:

$$\left(GM \frac{(h-z)}{(\Delta g - 2e)} \right)^{2/3} - (h-z)^2 < r^2$$

$$< \left(GM \frac{(h-z)}{(\Delta g + 2e)} \right)^{2/3} - (h-z)^2$$

Here G is the universal gravitational constant, Δg is the measured gravity change and e is the error associated with it. M and Δg are negative. The inequality defines spheroidal lobes of radius $r_{\min}$ and $r_{\max}$ beneath the station. Magma loss is permissible from any point between the two lobes. A three-dimensional envelope of lobes for all stations indicates which regions are permissible for magma loss (Fig. 2*b, c*). A relatively small region remains unforbidden. This is the region in which

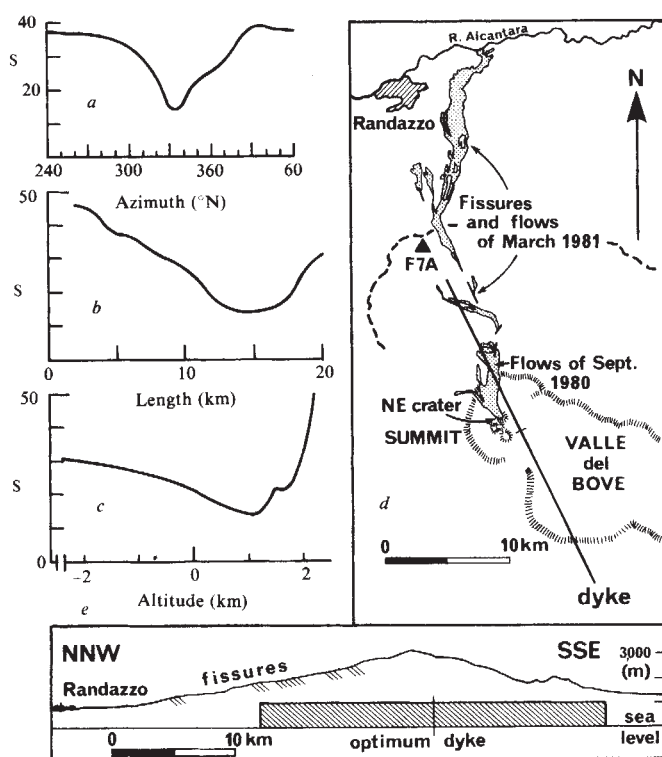


Fig. 3 Minimization of the statistic S against variation of azimuth (*a*), length (*b*) and depth (*c*) to upper surface of a dyke modelling the March 1981 eruption. The optimum dyke is shown in plan (*d*) and in section (*e*) relative to the eruptive fissures. (Map of fissures and flows from mapping by C. R. J. Kilburn (September 1980) and Guest *et al.* (March 1981).)

magma could have been stored before eruption, assuming that the observed changes were due to magma loss and that the loss was localized. The optimum point for magma loss can be found by minimizing residuals, and occurs at a point 2 km east and 4 km south of the summit. This model corresponds to the 60% probability level on a χ^2 test with 16 d.f., and therefore cannot be rejected. The fact that a point mass solution is possible, and that it lies so far south of the summit may be due partly to the strong southern bias of the stations available for survey.

Let us consider a more realistic model. We relax the point mass requirement and suppose instead a thin vertical dyke of variable size and position. Because no data are available for the northern summit region we assume the dyke to be symmetrical about the summit convergence of known historical fissure systems, which lies about 1.5 km east-southeast of the central crater (G. Kieffer, unpublished). The total mass of the dyke is set to 4.7×10^{10} kg and its vertical extent, which is not a sensitive parameter, is set to 1,000 m.

Modelling is performed using a computer program which models a dyke of any azimuth, length and depth as a lattice of point masses. For each model the sum of squared residuals, S , is calculated, and we search for an absolute minimum. A pronounced minimum exists, and its local behaviour is shown in Fig. 3a–c, where S is plotted against variation of each parameter. The optimum dyke is of azimuth $335^\circ \pm 5^\circ$, length $14 \text{ km} \pm 3$ and has its top surface at elevation $1,000 \text{ m} \pm 500$. For this dyke, $S = 14.1$, and it cannot be rejected at the 50% confidence level. Its position in plan and section is shown in Fig. 3d, e. It corresponds well with the surface activity of the March 1981 eruption.

A similar set of measurements was made immediately after the sub-terminal eruptions of September 1980. These occurred from the North-East Crater and were short-lived and highly explosive. The total mass of eruptive products is estimated at 2.8×10^{10} kg (C. R. J. Kilburn, personal communication).

No broad-scale gravity reduction was observed, and there were no reductions significant at the 95% level. Only one station, F9 to the north-east, showed a gravity reduction significant at the 68% level. Results are displayed, as before, in Table 1 and Fig. 1c. Stations on the south and south-east flanks showed a continuation of an upward trend which had been observed since 1979 and stations along the western flanks showed a pronounced increase. The increases were in the range $20\text{--}40 \mu\text{Gal}$. At the summit there was no significant change. In the absence of negative changes we cannot provide a simple model for the magma source; no model gives a fit which cannot be discounted at the 95% level. If the source is constrained to lie beneath the summit the statistic S improves as the source is lowered but never becomes acceptable. On this evidence the summit effusion of lava in September 1980 was not associated with a reservoir at detectable depth. It is proposed that the observed positive gravity changes were due to filling of radial dykes, associated with a sudden uprush of magma to the summit from a source well below sea level.

I conclude that these results support the view that Mount Etna underwent two distinct types of eruption during this period: a highly explosive summit eruption from a source well below sea level; and a massive flank eruption associated with draining from a radial dyke near sea level, which had become charged with magma during the preceding 6–12 months.

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Active *in situ* disaggregation of oceanic crust and mantle on Gorrige Bank: analogy with ophiolitic massives

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The Cyagor II cruise (May 1981) using submersible Cyana SP 3000 on Gorrige Bank off south-west Portugal confirmed the scheme proposed at the end of Cyagor I cruise^{1,2} that during the middle Cretaceous (110 Myr BP), Gorrige Bank, an old transform fault of Triassic–Liassic age, appears to have tilted to the north-east leading to the exposure of a section of oceanic mantle and crust. Due to its location at the boundary between European and African plates, Gorrige Bank suffered tectonic reactivation and uplifting during compressive and shearing-type events that occurred at the Cretaceous–Tertiary boundary and during the Tertiary (Eocene–Miocene). During the 12 dives of the Cyagor II cruise (Fig. 1), phenomena linked to the disintegration of serpentinites and gabbros in the deep-sea domain have been studied *in situ* with more detail than before³. Such processes have been suggested from studies on samples dredged or cored in oceans, or from observations in ophiolitic belts but direct observation had not enabled the magnitude of such phenomena to be measured. The observations on Gorrige Bank suggest that the processes described here are active in many oceanic sites. They help us to understand better the significance of many ophiolitic detritic beds more or less closely associated with pelagic sediments. We now compare the observations made at sea, especially on Gorrige Bank with similar observations in ophiolitic zones in mountain belts, which suggests that the ophiolite complexes have formed in a similar way to the breccias seen on the ocean floor.

Nine dives in areas where gabbros (Ormonde) and serpentinites (Gettysburg) outcrop revealed a very jagged deep-sea landscape made of small terraced cliffs. Each cliff, strongly fractured feeds a wide scree-covered slope. Fractures become more numerous at the top of the cliffs so that they are crowned by an unstable jumble of blocks. The size of the clasts released by the serpentine and gabbro disaggregation ranges from 1 mm to a few metres (Fig. 2).

Some large blocks of gabbro and serpentinite (between 1 and a few metres across) can be found a long way from any outcrop (100–1,000 m) where no other gabbroic or serpentinitic detritus occurs in the sediment. Under gabbro cliffs, two domains can be distinguished (Fig. 3): the first comprises $15^\circ\text{--}40^\circ$ slopes

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which are scree-covered and composed of heaps without any apparent order—small blocks, cobbles, pebbles mixed with coarse sands are all present. The absence of pelagic sediments on this scree reveals the incessant activity to which they are subjected. The second domain comprising 5°–15° slopes is an area of transition between the scree-covered slopes and the shelves covered with pelagic sediments—sand-banks scarcely covered with small blocks lay there. Sometimes sands form rhomboidal ripples moving downwards. These sand-ripples were often observed lying on pelagic sediments. These sands were found to be composed of pyroxene, monomineralic fragments of plagioclase and, less frequently, of dolerite rock fragments.

The serpentinite cliff feeds extremely varied scree with both decimetric blocks and gravels. At the foot of the scree, pelagic mud is strewn with small blocks and unjointed broken stones.

The *in situ* observations made on Gorringer Bank include those of active alteration processes in the deep-sea environment, as well as of erosion and transport of ophiolitic materials. The processes that were seen can be linked to different causes.

The first is the presence of rocky outcrops. The observations underline the multiplicity of escarpments, cliffs and fracture zones where gabbros and serpentinites are exposed. An intensive alteration is favoured by the internal fabric of the rocks themselves, particularly in the case of the gabbros. The alteration of granular type is linked with the crystallization of clays which are formed instead of plagioclases and then, release the grains of pyroxene. On the other hand, mechanical disaggregation takes place preferentially in the tectonized zone. In the case of Gorringer Massif, the known tectonic events^{1,2} date from Triassic–Liassic when the transform motion deduced from plate geometry was recorded by the development of shear zones in the gabbros and serpentinites.

This shearing of the compressive tectonics which are still affecting the Massif, as shown by the level of natural seismicity in the area and by first-motions studies¹⁶, has undoubtedly facilitated the creation of these highly tectonized zones. In the case of serpentinites, the tectonization plays a fundamental part in producing lenticular fracturing of the rock to give easily detached angular fragments of different sizes.

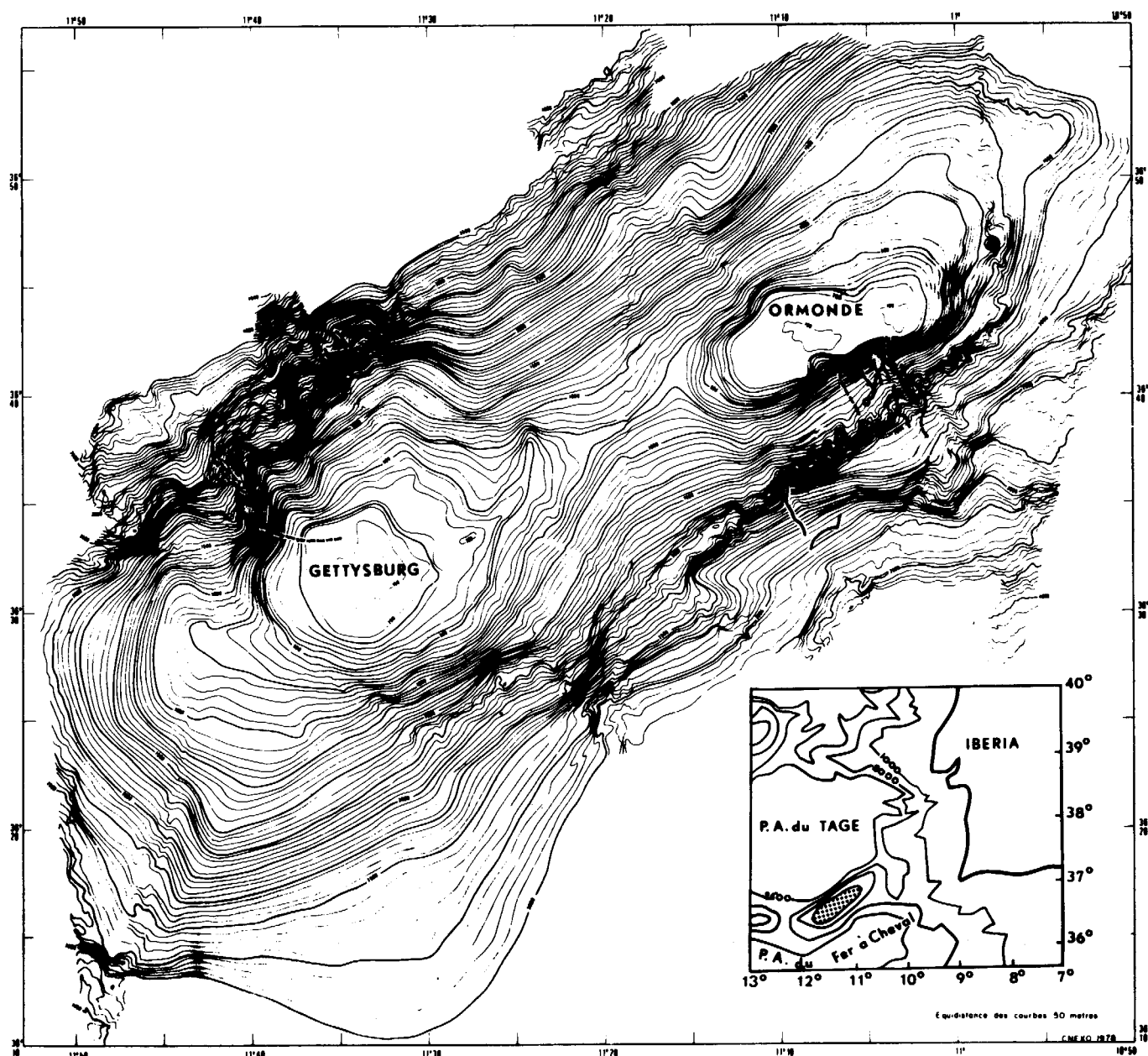


Fig. 1 Bathymetric map of Gorringer Bank with locations of *Cyana* SP 3000 dives. Solid lines, *Cyagor II* cruise 1981; dashed line, *Cyagor I* cruise 1977.

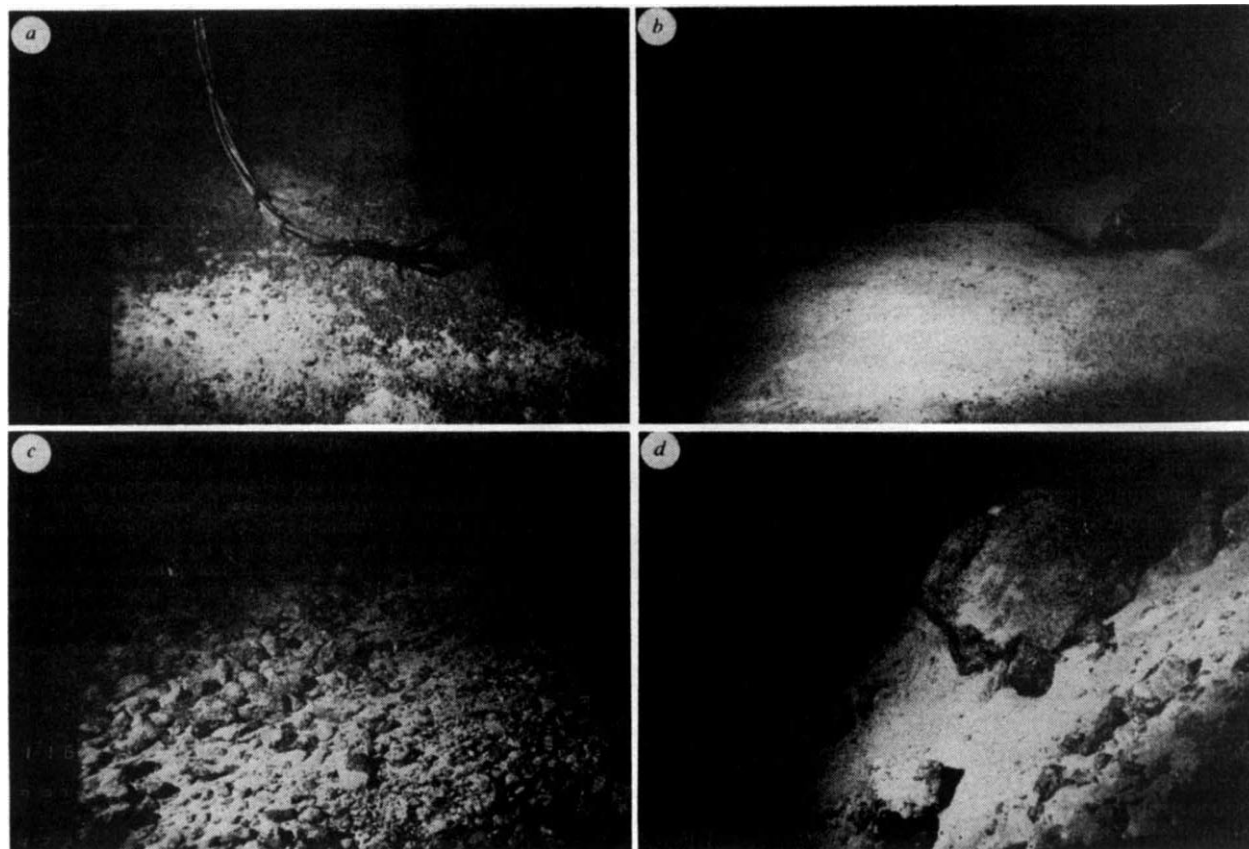


Fig. 2 Photographic plate: *a*, sands and gravels of gabbro; *b*, isolated block of gabbro; *c*, cobbles, pebbles and coarse sands of serpentinite; *d*, blocks of serpentinite in pelagic sediments.

The debris broken up by the double effect of granular alteration and tectonic disaggregation occupies a large area near the foot of the escarpment. Associated gravitational mass flows, distalites and coarse turbidites are bound to have participated in the filling of the adjacent abyssal plains.

Material, probably resulting from processes similar to those we have observed, has been located in many parts of the oceans, particularly in the proximity of complex structures involving a great variety of rocks of the mantle and of the oceanic crust such as mid-ocean ridges^{4,11,12,17-20}, fracture zones^{7,8,21-33}, and marginal basins³⁴⁻⁴². These materials are likely to be more important than previously estimated in the sedimentary series of oceanic basins.

Thus the genetic model obtained when studying oceanic data leads us to consider that any outcrop of the oceanic crust, whether tectonized or not, supplies detrital material. Most of this material remains *in situ* at the foot of the cliffs. The remainder is scattered in the surrounding autochthonous sedimentation mainly in two forms: metre-sized blocks which may slide down the slopes, and fine grained or coarse sand transported by turbidity currents. This latter fraction is consequently included in diversified and genetically different formations.

In mountain belts two types of deposits may be found for ophiolitic detrital products; *in situ*, that is to say closely associated with large igneous ophiolitic bodies, and scattered in the metasedimentary series^{13-15,43-51}. These series may form independent tectonic units, so that the ophiolitic detrital material they contained will seem totally disconnected from its sources.

One can note a striking analogy between old and present day oceanic crust detritism found in the ocean and ophiolitic debris found in the mountain belts. However, we conclude that these types of detritic formations probably occupy a significant place in the total volume of ancient and present day oceanic sediments.

The results obtained in the ocean and particularly the *in situ* observations in the Gorringe Massif enable us to specify the morpho-structural framework of these present day and ancient deposits (cliffs, rugged escarpments, small basins). In all known cases in the oceans these structures belong or have belonged to areas topographically high in comparison with the oceanic floor (fracture zones, ridges, aseismic ridges). Thus, when these detritic levels are found in the chains, one must wonder if there is a relation between their genesis and the presence in the old ocean (Tethys Ocean, for example) of structures of abnormal topography. Those structures could result from an early intra

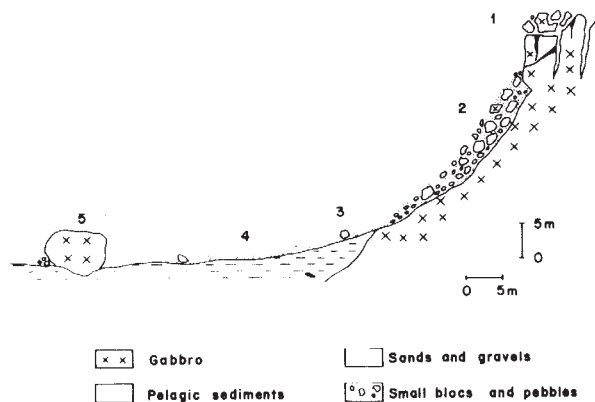


Fig. 3 *In situ* observations on Gorringe Bank (Mont Ormonde). 1, Highly fractured bedrock; 2, unbedded blocks, pebbles and sand at the foot of an escarpment; 3, small blocks isolated in sand and gravels; 4, sand and gravel ripples; 5, block of metric size.

oceanic tectonic event (as on Gorringle, for example) and, despite variable causes, could be constant.

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Spherulitic crystallization in lamprophyric magmas and the origin of ocelli

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Ocelli are rounded structures containing polycrystalline feldspar aggregates and minor amounts of mafic minerals; they occur in lamprophyre dykes all over the world. Ocelli are usually interpreted in terms of liquid immiscibility^{1–3}. In alkaline lamprophyre dykes along the south coast of Norway⁴, ocelli are closely associated with globular structures (varioles) which I have assumed⁵ were formed as a result of a splitting of the magma into two fractions. The re-examination of these Norwegian dykes reported here, however, has shown that varioles, ocelli and leucocratic feldspar-rich segregations formed by spherulitic and closely related growth mechanisms from under-cooled volatile-rich magmas without an intervening stage of liquid phase separation.

The morphology of the crystalline aggregates varies widely and is related to the distance from the dyke margins. The dykes nearly lie flat and never exceed 2 m in thickness. Depending on the mode of crystallization three different zones can be distinguished (Fig. 1). (1) A marginal zone, including a chill zone, characterized by globular growth units. Mutual impingement of the globules has here resulted in the formation of a polyhedral cell structure (Fig. 2). Dykes <30 cm thick are globular throughout. The thickness of the marginal zone in the larger dykes is 10–15 cm. The globules exhibit features characteristic of spherulitic growth. (2) An intermediate zone containing feldspatic segregations. This zone is only present in the upper part of dykes >50 cm thick. (3) A central homogeneous zone.

A very fine-grained chilled zone, 2–3 mm thick, is usually present in the marginal zone. The chilled zone contains many globules, the size of which is remarkably uniform and ~0.1 mm in diameter. In polarized light, each globule shows a black cross demonstrating that crystal fibres radiate in all directions from a central nucleation point. Microphenocrysts of kaersutite, titaniferous magnetite and augite are commonly enclosed in

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the globules. Kaersutite and augite are arranged with their long dimensions parallel to the dyke walls. The globules are usually arranged in long sub-parallel chains or layers parallel to the dyke margins presumably reflecting nucleation and growth along flow lines in the magma.

The size of the globules and the polyhedral cells, hereafter termed spherulites, increases away from the chilled zone and may become 10–12 mm in diameter 10–15 cm from the contact. The spherulites are essentially composed of alkali-feldspar, kaersutite, ±augite and Ti-magnetite. The feldspar forms elongate, curved and branching crystals radiating from a central point. However, with increasing grain size inwards from the dyke margins it often becomes impossible to recognize radiate crystal arrays because any section which does not intersect the centre of the spherulite appears to consist of a granular crystalline aggregate.

The polyhedral cells are separated by a layer of a greenish material, which sometimes contains pyrite. The thickness of this layer is <10 µm in the chill zone and increases to ~100 µm in the dyke marginal zone.

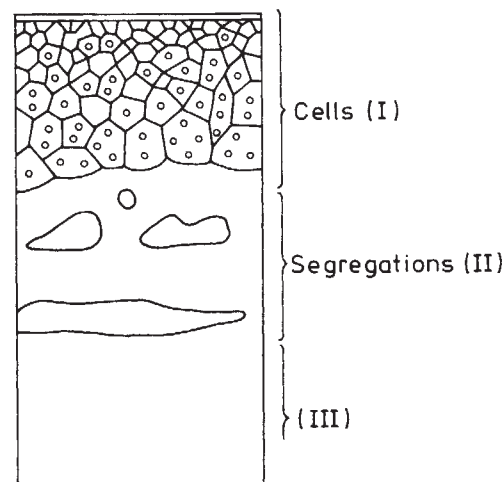


Fig. 1 Development of cells and leucocratic segregations in the marginal (I) and intermediate (II) zones respectively. Ocelli are indicated by circles. Not drawn to scale.

Except for the finest compact spherulites of the chill zone, the spherulites always contain some rounded patches consisting almost exclusively of alkali feldspar. In each patch the crystals radiate from a common centre (Fig. 3a), and inwards in the dykes there is a change from a fibrous to a more lath-shaped morphology. These cone-shaped growth units (fan spherulites) apparently nucleated along the growth front of the larger spherulites in which they occur. It is frequently observed that they have grown from side-branches of radiating feldspar crystals (Fig. 3b). The fan spherulites actually represent the ocelli of the earlier description⁴, which imposes constraints on the formation of the features. The observations preclude the possibility that the ocelli separated as liquid droplets.

With increasing distance from the dyke walls the polyhedral cell structure gradually disappears. This is due to a transition in morphology of the spherulites from compact to open, in the terminology of Keith and Padden⁶. When two open spherulites meet during growth the lamellae from both extend across the 'boundary' producing an interleaving structure.

The leucocratic segregations⁴ in the intermediate zone are closely related to the ocelli of the marginal zone. The most abundant phase, alkali feldspar, shows a marked tendency to occur in thin bands or lenses roughly parallel to the dyke walls. Some bands consist of open, coarse spherulites, others consist of curved, branching feldspar crystals with preferred elongation inwards forming a comb-layer^{7,8}. Kaersutite, which comes next in abundance, does not appear to grow directionally. The lamprophyre itself is very rich in kaersutite and Ti-magnetite along the upper and lower boundary of the segregations. The segregations contain scattered rounded ocellar structures, consisting of superimposed feldspar lamellae which are attached to one another like pages in an open book.

Chilled zones arise when the magma, coming in contact with the cold walls of the host rock, becomes greatly undercooled by the high rates of heat extraction. The higher the cooling rates, the lower the temperature at which nucleation occurs. Spherulitic growth is generally associated with rapid cooling rates⁹, and chill zones would thus be an ideal environment for the growth of spherulites. The withdrawal of heat formed thermal gradients normal to the dyke walls, and crystallization proceeded from the margin inwards. The increasing size of the polyhedral cells away from the contact shows that the rate of nucleation decreased in the same direction. The growth of the spherulites approached a steady state and solidification terminated by the impingement of adjacent spherulites. The mafic sulphide-rich boundary layer which separates the cells has resulted simply from the impingement of enveloping boundary layers of rejected elements advancing ahead of the outer boundary of the spherulites. The thickness of the layer should be of the order of twice the characteristic distance of the boundary layer or $2D/G$ where D is the diffusion coefficient and G the growth rate⁶. Assuming, for example, a diffusion coefficient of $10^{-7} \text{ cm}^2 \text{ s}^{-1}$, the growth rate of the spherulites in the chill zone would be of the order of 0.1 mm min^{-1} .

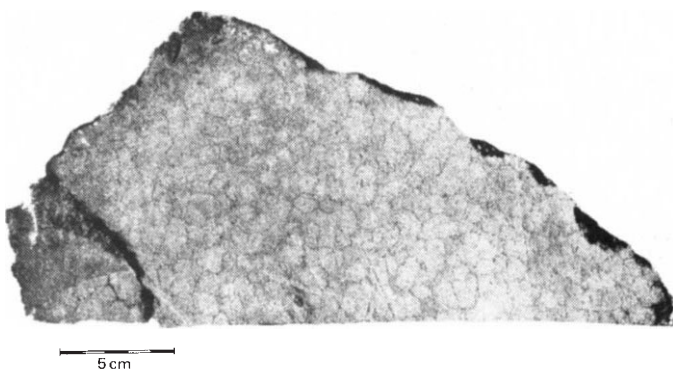


Fig. 2 Weathered specimen showing polyhedral cells. The structure is never observed on fresh surfaces, but may be developed by etching. The ocelli are 0.5–1 mm in diameter.

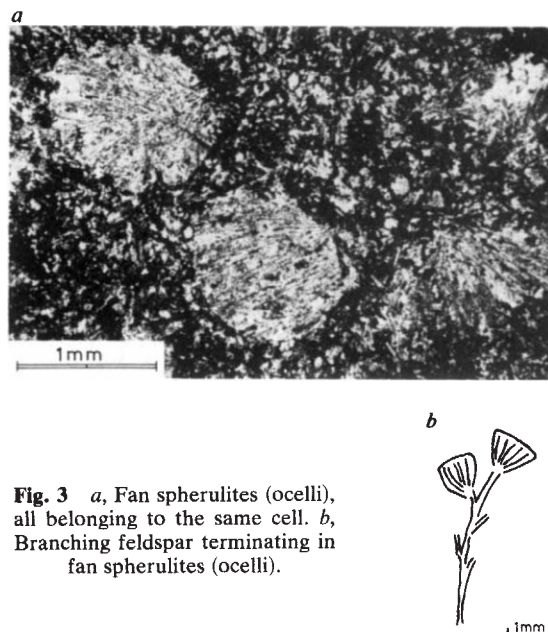


Fig. 3 a, Fan spherulites (ocelli), all belonging to the same cell. b, Branching feldspar terminating in fan spherulites (ocelli).

The textural developments seem to be related to feldspar nucleation difficulties in a lamprophyric magma, and the crystallization sequence is considered to have been determined by the relative ease of nucleation of the minerals involved rather than by equilibrium phase relationships. Delayed nucleation of feldspar is common in systems varying in composition from granitic to basaltic⁹ presumably because of a slow rate of formation of embryonic clusters of equilibrium size. Thus, feldspar phenocrysts are always absent in lamprophyres and the fundamental shape of the feldspar is frequently spherulitic/dendritic indicating crystallization from an undercooled liquid. In the marginal zone, the high rate of cooling produced undercoolings of sufficient magnitude to permit the nucleation of feldspar simultaneously with the mafic minerals with the result that spherulites of melt composition grew and formed a cell structure. In the intermediate zone, slower cooling rates induced early nucleation of the mafic minerals relative to feldspar. The growth of kaersutite and magnetite established composition gradients in the melt perpendicular to the dyke walls. The melt was exposed to an increasing degree of undercooling by loss of water and iron to crystallizing kaersutite and ore minerals, and when feldspar finally did nucleate, conditions were favourable for the growth of feldspar-rich spherulites or alternatively inward growth of fan-shaped bundles of feldspar. The leucocratic segregations thus formed by periodic build-up of appropriate conditions of undercooling.

Regardless of the detailed mechanisms involved, the present study has shown that the globules (varioles), the polyhedral cells, the leucocratic segregations, and the ocelli which occur in them, are polycrystalline aggregates formed by crystal growth, the structural variations being a kinetic effect related to cooling rates. The observations presented here have produced no evidence in favour of liquid–liquid phase separation, although this still remains a possible explanation for the formation of other ocellar structures.

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Isolation of a natural hatching stimulus, glycinoeclepin A, for the soybean cyst nematode

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The cyst nematodes (genera *Heterodera*, *Globodera* and others)¹ generally have a limited host range. This specificity arises from the fact that the hatching of larvae from cysts occurs in response to specific stimuli secreted by the host plants²⁻⁴. Because of the scientific interest and importance in agriculture of these stimuli, since their first recognition⁵ in 1922 much time and effort²⁻⁴ has been expended in attempts to purify and characterize them. However, success has been limited by the extremely small amounts of active substance available. We report here the isolation of a natural hatching stimulus for the soybean cyst nematode from the roots of kidney bean. The substance, designated as glycinoeclepin A, stimulates the hatching of larvae from the eggs *in vitro* at a concentration of 10^{-11} to 10^{-12} g ml⁻¹ in water at 25 °C.

The soybean cyst nematode (*Heterodera glycines* Ichinohe) parasitizes a small group of host plants, including soybean (*Glycine max*), kidney bean (*Phaseolus vulgaris*) and adzuki bean (*Phaseolus angularis*), and causes 'soybean sickness'⁶. Tsutsumi and Sakurai⁷ demonstrated that the hatching of larvae from cysts of the nematode was stimulated by root diffusates from the host plants. Since then various factors affecting hatching have been examined in detail⁸⁻¹¹. The present investigation is based on these findings.

The bioassay for hatching was performed with free eggs. The cysts were isolated from infected soil collected in October at Memuro, Hokkaido, and stored in a refrigerator at 5 °C. Before the test, the cysts were soaked in water at 25 °C for 10 days, then dissected in water and sieved through a 325-mesh sieve. The dissected cyst wall fragments remained on the sieve. The eggs and larvae were transferred to a beaker of water so that 1 ml of the aqueous suspension contained 150–200 eggs. Then 1 ml of the suspension was transferred to a Syracuse watch glass, to which 8 ml of distilled water and 1 ml of the test solution were added. The whole suspension was kept at 25 °C and the number of the larvae hatched was counted after 10 days. The per cent hatching rate was expressed as $P = P_t - P_w$, where P_t and P_w denote the per cent hatching observed in the

test solution and in distilled water, respectively. The test solution was said to be active when the hatching rate, P , exceeded 50%. Hatching rate is an adequate measure of hatching and emergence⁷.

Hatch-stimulating concentrates were prepared from aqueous extracts of the roots of two subspecies ('Hon-kintoki' and 'Beni-kintoki') of kidney bean rather than from their root diffusates. Dried and powdered roots (113 kg) of Hon-kintoki, collected in late July at Memuro (1 hectare), were extracted with water at a temperature of <10 °C, and the aqueous suspension, after concentration at <30 °C, was acidified to pH 2–3 and extracted again, with chloroform. The chloroform solution gave 'chloroform extracts A' (102 g), which were fractionated according to the procedure summarized in Table 1, guided by the bioassay. Repeated chromatography yielded a highly active fraction, fraction L (~50 µg). Dried roots of Beni-kintoki were also treated and fractionated in almost the same manner to give the corresponding fraction with the same activity. These fractions were homogeneous and identical by analytical HPLC over various columns (µ-Bondapak NH₂, MicroPak NH₂-10,

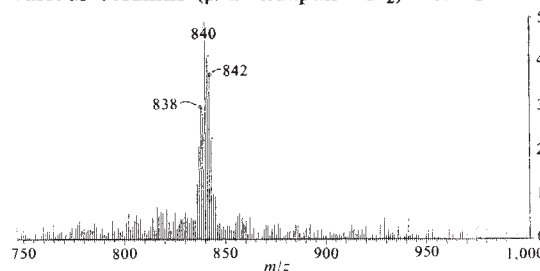


Fig. 1 FD mass spectrum of *p*-BPE of glycinoeclepin A.

µ-Bondapak C₁₈ and µ-Bondapak/Phenyl) and by the respective field desorption (FD) mass spectra (Fig. 1). The identification of glycinoeclepin A as the active substance was also checked by the division of fraction L into five fractions by chromatography on two separate columns (µ-Bondapak NH₂ and µ-Bondapak C₁₈) followed by the bioassay of each fraction after hydrolysis.

The FD mass spectrum (Fig. 1) established that the bis(*p*-bromophenacyl) ester of glycinoeclepin A (GEA *p*-BPE) has a molecular weight of 838 and hence that glycinoeclepin A is a dibasic acid with the molecular weight of 446. The high-resolution FD mass spectrum ($m/z = 838.1334$) indicated that GEA *p*-BPE is best represented by C₄₁H₄₄O₉Br₂ (calculated $m/z = 838.1352$) and hence the acid by C₂₅H₃₄O₇. This was supported by the ¹H-NMR spectrum (Fig. 2) of GEA *p*-BPE, which revealed about 34 hydrogen and 7 oxygen atoms (two —COOH, one —OH, one —O— and one —C=O) in the acid.

Table 1 Isolation of glycinoeclepin A as its bis(*p*-bromophenacyl) ester

Dried and powdered roots of kidney bean 'Hon-kintoki' (113 kg)
↓ in the text
Chloroform extracts A (102 g, active at 10^{-5} g ml ⁻¹)
↓ EtOAc-aq. NaHCO ₃
Acidic extracts B (57 g, active at 10^{-5} to 10^{-6} g ml ⁻¹)
↓ Charcoal-Celite column (aq. acetone)
Fraction C (21.5 g, active at 10^{-6} g ml ⁻¹)
↓ Sephadex LH-20 column (MeOH)
Fraction D (10.1 g, active at 10^{-6} to 10^{-7} g ml ⁻¹)
↓ DEAP-Sephadex LH-20 column (72% EtOH-AcOH-NH ₄ OH)
Fraction E (2.16 g, active at 10^{-7} g ml ⁻¹)
↓ Sephadex LH-20 column (CH ₂ Cl ₂ -MeOH, 95:5)
Fraction F (135 mg, active at 10^{-8} g ml ⁻¹)
↓ <i>p</i> -Bromophenacylation (<i>p</i> -BrC ₆ H ₄ COCH ₂ Br and (i-Pr) ₂ NEt in MeCN, room temperature, overnight) and silica gel column
Fraction G as <i>p</i> -BPE (316 mg, active at 10^{-8} g ml ⁻¹ after hydrolysis)
↓ Preparative HPLC, Hitachi Gel 3019 column (MeOH-CH ₂ Cl ₂ , 7:3)
Fraction H as <i>p</i> -BPE (112 mg, active at 10^{-8} to 10^{-9} g ml ⁻¹ after hydrolysis)
↓ Preparative HPLC, Bondapak C ₁₈ /Porasil B (MeOH-H ₂ O, 8:2)
Fraction I as <i>p</i> -BPE (29 mg, active at 10^{-9} g ml ⁻¹ after hydrolysis)
↓ Preparative HPLC, µ-Bondapak NH ₂ column (C ₆ H ₁₄ -CH ₂ Cl ₂ -MeCN, 70:18:12)
Fraction J as <i>p</i> -BPE (2.2 mg, active at 10^{-10} g ml ⁻¹ after hydrolysis)
↓ Preparative HPLC, µ-Bondapak NH ₂ column (C ₆ H ₁₄ -CH ₂ Cl ₂ -MeCN, 80:12:8)
Fraction K as <i>p</i> -BPE (1.2 mg, active at 10^{-10} g ml ⁻¹ after hydrolysis)
↓ Preparative HPLC, µ-Bondapak NH ₂ column (C ₆ H ₁₄ -CH ₂ Cl ₂ -MeCN, 60:36:4)
Fraction L (glycinoeclepin A) as <i>p</i> -BPE (~50 µg, active at 10^{-11} to 10^{-12} g ml ⁻¹ after hydrolysis)

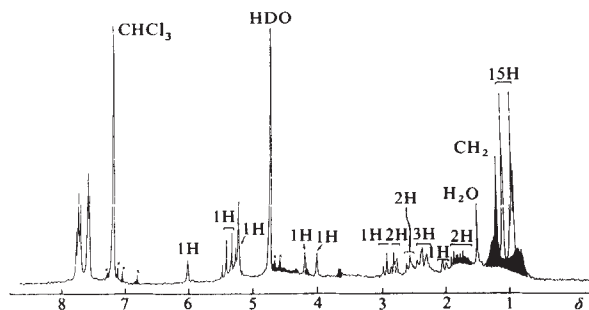


Fig. 2 ^1H -NMR spectrum of *p*-BPE of glycinoeclepin A (270 MHz, 50 μg in $\text{CDCl}_3 + \text{D}_2\text{O}$). 12,260 scans.

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Long-term potentiation of the perforant path *in vivo* is associated with increased glutamate release

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Long-term potentiation (LTP) of synaptic transmission following brief trains of high-frequency stimulation in hippocampal pathways has attracted attention as a possible physiological correlate of memory. The mechanism of LTP is little understood, and what evidence there is suggests that both pre- and postsynaptic mechanisms are involved^{1–7}. Previous findings that Ca^{2+} is required for LTP^{8,9}, and that LTP is accompanied by a prolonged increase in Ca^{2+} uptake¹⁰, suggest that an increase in impulse-dependent release of transmitter, a process critically dependent on presynaptic Ca^{2+} levels^{11,12}, may be an important component of this form of synaptic plasticity. A recent report¹³ that release of previously accumulated ^3H -D-aspartate is increased following tetanic stimulation of afferent fibres to CA1 pyramidal cells in hippocampal slices is also consistent with a presynaptic mechanism. However, there has been no direct evidence relating LTP to an increased stimulus-dependent release of transmitter. We have now investigated whether LTP in the perforant path (PP) of the rat is associated with a change in the release of neurotransmitter. A modification of the push-pull cannula technique has enabled us to infuse ^3H -glutamine and measure the subsequent release of newly synthesized ^3H -glutamate, and at the same time to monitor the field potentials evoked in the dentate gyrus by stimulation of the PP. We have found a prolonged increase in the release of glutamate, the probable transmitter of the PP^{14,15}, following the induction of LTP in this pathway.

In anaesthetized rats, a push-pull cannula was placed in the molecular layer of the dentate gyrus, where PP fibres terminate on the apical dendrites of granule cells. Two insulated stainless-steel wire recording electrodes were attached to the outer cannula so that the exposed tip of the upper electrode lay roughly midway between the ends of the inner and outer cannulae, and the lower electrode extended 0.2–0.4 mm beyond the inner cannula (Fig. 1a). Correct placement of the electrode/cannula assembly was achieved by lowering it to a depth at which the negative-going potential evoked by stimulation of the PP was maximal, as recorded through the upper (dendritic) electrode. The potential recorded through the lower (somatic) electrode, positioned in the granule cell body layer, was used to monitor synaptic transmission during the experiment; the amplitude of the initial positive-going component of this response, which we refer to as the synaptic wave (Fig. 1a), was used as a measure of the strength of synaptic activation¹⁶. Following the induction of LTP by a high-frequency stimulus train (see Fig. 1 legend) an increase in the amplitude of the synaptic wave normally occurs (Fig. 1a), persisting with little decrement after the first 15 min (Fig. 1b, high-frequency train delivered at the time indicated by the arrow, mean of 11 animals showing LTP).

To investigate the release of glutamate in the dentate gyrus, the area to be perfused was preloaded with the radiolabelled precursor, ^3H -glutamine. Because the enzyme glutaminase, which converts glutamine to glutamate, is concentrated in pre-synaptic terminals¹⁷, most of the infused ^3H -glutamine is probably taken up by the glutamate-utilizing terminals of the PP, rather than in postsynaptic or non-neuronal elements^{18–20}. ^3H -glutamine (20 μCi) was infused through the inner cannula over 30 min. One hour later, perfusion with oxygenated medium was begun. The ^3H -glutamate present in sequential fractions of the perfusate was separated and measured as described in Fig. 2 legend.

In control experiments, the release of ^3H -glutamate into the perfusate while the PP was stimulated continuously at 0.1 Hz showed a steady decline during the perfusion (Fig. 2a). The release of ^3H -glutamate was at least partially stimulus dependent, since in seven animals, when the rate of stimulation was increased 30-fold, from 0.1 to 3 Hz, and the stimulus voltage was doubled, 120 min after the start of perfusion, the release of ^3H -glutamate was markedly increased (Fig. 2b). Note, however, that although larger potentials were recorded with no decrement throughout this 75-min period of increased stimulation, the mean outflow of ^3H -glutamate returned to a level not significantly greater than control values within 45 min, possibly due to depletion of the pool of ^3H -glutamate.

In another set of animals, the pattern of ^3H -glutamate release following the induction of LTP was investigated. The PP was stimulated at 0.1 Hz throughout the experiment, and superimposed on this, a high-frequency train (250 Hz for 0.5 s, stimulus voltage doubled) was delivered to the PP 120 min after the start of perfusion (arrows in Figs 1b, 2c and d). LTP of the PP-granule cell synapse (as judged by an increase in the amplitude of the synaptic wave lasting for 30 min or more) was produced in 11 out of 17 animals. The mean time course of potentiation is shown in Fig. 1b for all the animals in which LTP occurred. All these animals showed a prolonged increase in the release of ^3H -glutamate after the induction of LTP; indeed, the average level of released ^3H -glutamate in the potentiated animals remained above the corresponding average control value until the end of the experiment, 75 min later (Fig. 2d). In contrast, the six animals which received a high-frequency train, but in which the criteria for LTP were not met, showed only a slight elevation of ^3H -glutamate release following the train compared with control animals (Fig. 2c). As a further control that the prolonged increase in ^3H -glutamate release shown in Fig. 2d was associated with the events following the high-frequency conditioning train rather than the train itself, the same number and strength of stimuli used to produce LTP were given at a lower frequency (2 Hz) to five animals, 120 min

Fig. 1 Long-term potentiation in the perforant path during perfusion of the dentate gyrus through a push-pull cannula. Experiments were performed on male Sprague-Dawley rats (300–330 g), anaesthetized with urethane (1.5 g kg^{-1} , intraperitoneally). *a*, Two recording electrodes (insulated stainless-steel wire, 0.1 mm o.d.) were glued to the outer ('pull') tube of the concentric push-pull cannula (0.83 mm o.d., Plastic Products, Clark Electromedical). The upper electrode protruded 0.2 mm, and the lower electrode 0.5–0.7 mm below the outer cannula. The electrode/cannula assembly was lowered stereotactically into the dentate gyrus of the hippocampus. The outer cannula was fixed to the skull with dental cement, and the inner ('push') cannula was then lowered so that its tip protruded 0.3 mm below the outer cannula. The PP was stimulated through a concentric bipolar stimulating electrode (Rhodes, Clark Electrochemical) with constant voltage pulses (0.05 ms, range 10–50 V) at a strength near threshold for a population spike (the negative-going spike superimposed on the synaptic wave and reflecting the discharge of granule cells). Throughout the experiment, evoked potentials were recorded every 90 s on a chart recorder, and on disc for subsequent computer analysis. Examples are shown of potentials recorded from the upper (dendritic) and lower (somatic) electrodes during perfusion in a single animal, immediately before and 30 min after a high-frequency (h.f.) stimulus train (250 Hz, 0.5 s, stimulus voltage doubled). *b*, Graph showing the mean percentage increase ($\pm 1 \text{ s.e.m.}$) in the amplitude of the synaptic wave for all animals ($n = 11$) which displayed LTP after receiving a high-frequency conditioning train 120 min after the onset of perfusion. In each animal the amplitude of the synaptic wave was measured at a fixed latency and expressed as a percentage of its mean amplitude during the 30 min preceding the conditioning train.

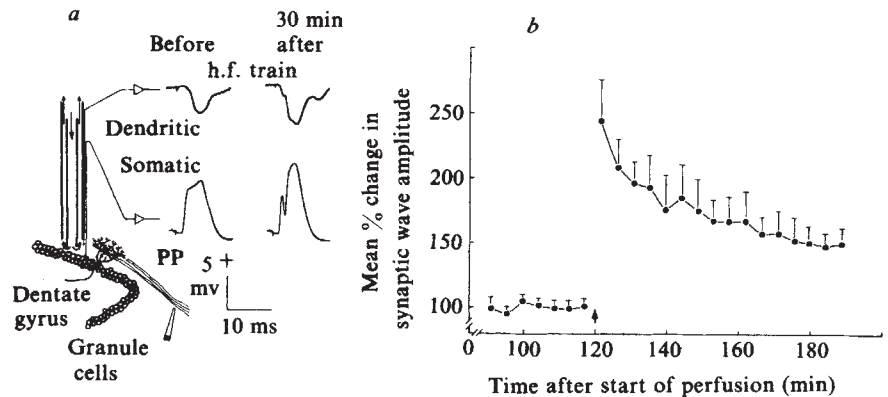
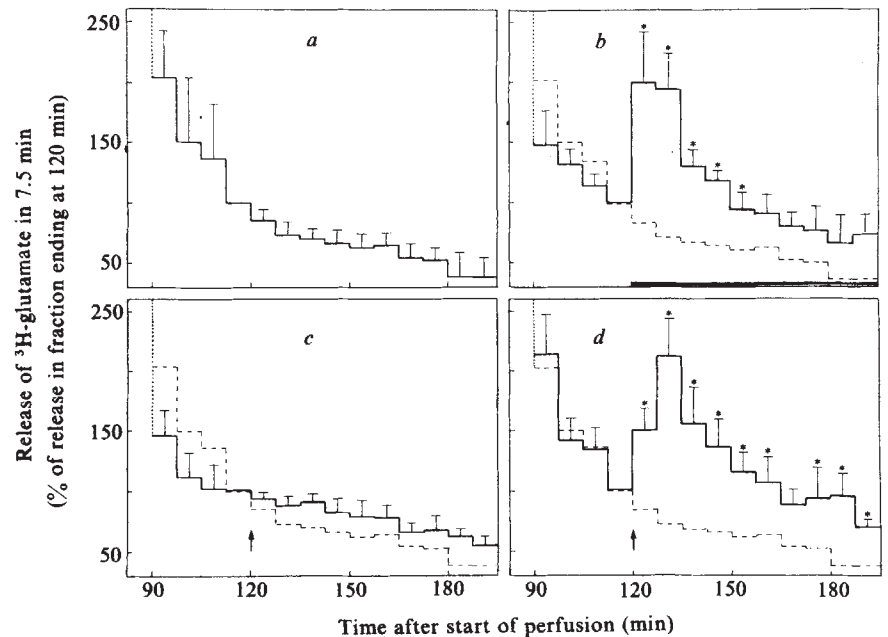


Fig. 2 *In vivo* release of ^3H -glutamate from the dentate gyrus perfused through a push-pull cannula. In all experiments ^3H -glutamine ($20 \mu\text{Ci}$, 0.75 nmol) was infused in $3 \mu\text{l}$ oxygenated ($95\% \text{ O}_2/5\% \text{ CO}_2$) perfusion medium (composition in mM: Na^+ , 152; K^+ , 3.7; Mg^{2+} , 1.2; Ca^{2+} , 1.8; Cl^- , 140; H_2PO_4^- , 1.2; SO_4^{2-} , 1.2; HCO_3^- , 16; glucose, 10) containing a trace of phenol red, at a rate of $0.1 \mu\text{l min}^{-1}$. Perfusion with continuously oxygenated medium at $10 \mu\text{l min}^{-1}$ was begun 1 h later, using a dual-channel peristaltic pump. Sequential 7.5-min fractions of perfusate were collected on dry ice. The phenol red served to mark the entry of the perfusate into the fraction collector, and thus the lag time of the apparatus (8 min). ^3H -glutamate was separated from ^3H - γ -aminobutyric acid and from their precursor ^3H -glutamine as previously described^{21,22} using anion-exchange columns. The ^3H -glutamate released in a single experiment was expressed for each fraction as a percentage of the release in the fraction ending at 120 min. Values for each group of animals are given as mean $\pm 1 \text{ s.e.m.}$ for all fractions between 90 and 195 min following the start of perfusion. *a*, Control perfusion, PP stimulated continuously at 0.1 Hz ($n = 7$). Values for control ^3H -glutamate release are repeated in *b*, *c* and *d* (dashed lines), where * indicates significant increase in ^3H -glutamate release compared with control ($P < 0.05$, Student's *t*-test). *b*, PP stimulated at 0.1 Hz until 120 min after start of perfusion when the rate was increased to 3 Hz and the stimulus strength was doubled for the remainder of the experiment as indicated by the solid bar ($n = 7$). *c*, *d*, PP stimulated continuously at 0.1 Hz, and at 120 min after the start of perfusion (arrow) a conditioning train was given (see Fig. 1 legend). *c*, ^3H -glutamate release in animals not showing LTP ($n = 6$). *d*, ^3H -glutamate release in animals showing LTP ($n = 11$).



after the start of perfusion. No potentiation occurred, and there was no difference in ^3H -glutamate release relative to controls, apart from a small increase in the fraction corresponding to the low-frequency train (results not shown).

Much evidence points to glutamate as having a transmitter role in the PP^{14,15}. Our finding of a stimulus-dependent release of glutamate in this pathway (Fig. 2b) provides additional evidence for such a role. The main finding here, that LTP is associated with a prolonged increase in ^3H -glutamate release thus strongly suggests that a sustained increase in stimulus-dependent release of this transmitter is at least one of the mechanisms underlying LTP. This does not rule out the possibility that other changes involving, for instance, increased dendritic uptake of Ca^{2+} (ref. 10), altered dendritic morphology⁴, or other mechanisms leading to an enhanced postsynaptic sensitivity⁵ also contribute to LTP. The relative roles of pre- and

postsynaptic events in the long-term enhancement of synaptic efficacy remain to be determined.

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Reversal of current through calcium channels in dialysed single heart cells

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Calcium channels contribute to important cellular functions such as impulse conduction, rhythmic activity, muscle contraction, and secretion¹ and are a major target for modulation by neurohormones and drugs^{2,3}. Despite their importance, Ca channels are much less well understood than Na and K channels because of several problems reviewed by Hagiwara and Byerly⁴. First, few preparations allow good control of electrical potential and ionic composition on both sides of the membrane, yet display strong Ca currents. Second, current carried by Ca channels is often obscured by ion movements through other membrane pathways. Third, outward current flow through Ca channels, carried by Ca^{2+} or any other ion, has been difficult to demonstrate. This is unfortunate because measurements of the potential at which current reverses (E_{rev}) have been crucial in understanding ion permeation and selectivity in other ionic channels (see ref. 4). In molluscan neurones, attempts at recording outward current through the Ca channel have not succeeded, largely because of overlap by nonspecific outward current^{5–7}. In multicellular cardiac muscle preparations, strong depolarizations produce a decaying outward current that Reuter and Scholz attributed to K efflux through the Ca channel⁸. Their interpretation is controversial, however, since the evidence leaves open other explanations for the outward current—for example, Na efflux via Na–Ca exchange⁹, or K efflux through K-selective channels. To overcome these problems, we studied Ca channels in single isolated heart muscle cells^{10–12} using a suction pipette method¹³. We were able to record robust Ca currents with minimal interference from other time-dependent currents while controlling potential and ion composition on both sides of the membrane. Here we present experimental evidence for a genuine reversal of ionic current through Ca channels due to outward movement of K^+ ions, in support of the hypothesis of Reuter and Scholz.

Single isolated cells were obtained from the ventricles of adult guinea pig hearts by enzymatic dissociation using collagenase and hyaluronidase. Relaxed cells with cylindrical geometry were chosen for study; in a separate series of micro-electrode recordings, such cells displayed resting potentials near –80 mV, vigorous contractions, and action potentials with full plateaus. A suction pipette (tip diameter 5–10 μm) provided access to the inside of the cell for voltage clamp and internal dialysis¹³. The internal dialysis fluid contained (in mM): 75.5 K-aspartate, 29 K_2HPO_4 , 17.5 KH_2PO_4 , 5.5 glucose (pH 7.0, pCa 6.4). In some experiments (Fig. 2a, b), potassium salts were replaced by caesium salts. Except where otherwise noted, the external solution contained (in mM): 137 NaCl, 11.9 NaHCO_3 , 0.42 NaH_2PO_4 , 5.4 KCl, 1.05 MgCl_2 , 1.0 CaCl_2 , 5.5 glucose and 10 μM tetrodotoxin (TTX, pH 7.4). In these solutions, the resistance of the suction pipette tip was usually 0.8–1.2 M Ω , and the magnitude of the peak inward Ca current was a few nanoamperes. The resulting ohmic drop of a few millivolts was offset by series resistance compensation. Transmembrane voltage and current signals were recorded and analysed using a

Digital Equipment 11/03 minicomputer. All experiments were done at room temperature (21 °C).

In all experiments, the membrane potential was held at –40 mV to inactivate any Na channels not blocked by 10 μM TTX. Figure 1 shows membrane currents associated with a series of 150-ms clamp pulses, spaced 10 mV apart. The magnitude of the peak inward current increases gradually with depolarization up to 0 mV as more and more Ca channels become activated (a). With pulses above +10 mV (Fig. 1b), the time-dependent inward current diminishes and eventually disappears at +70 mV. Beyond this apparent reversal potential, E_{rev} , the time-dependent current becomes increasingly outward. This pattern of current inversion was found consistently in each of the 70 cells we examined; with 1 mM Ca^{2+} outside and 150 mM K^+ inside, E_{rev} was always found near +60 or +70 mV.

Figure 1b illustrates the inversion of time-dependent current over the time scale of Ca channel inactivation. If the reversal is a property of current flow through Ca channels themselves, it should also be evident during the first few milliseconds following a clamp step, as the Ca channels open. Figure 1c, d shows records taken at a fast sampling rate in another single cell. The traces show membrane current after subtraction of linear capacity and 'leak' currents. Activation of inward Ca current becomes increasingly rapid over the range between –30 and +10 mV (c). This is in qualitative agreement with previous work on multicellular preparations (for example, ref. 8), but at all potentials the time-to-peak Ca current is briefer in these single cells. The family of records in panel d shows the behaviour at more positive potentials. The time-dependent current is maximally inward at +20 mV, virtually absent at +75 mV, and increasingly outward beyond this E_{rev} . Thus, the records are consistent with a true reversal of Ca channel current.

The ionic nature of the outward current at strongly positive potentials was explored by varying the ionic composition of the external or internal solutions. The outward current cannot be attributed to Ca efflux because intracellular free Ca^{2+} was kept low by the internal anions, and in some experiments (Fig. 2a), by internal EGTA as well. (The effectiveness of the Ca^{2+} buffering was indicated by the absence of contractile activity even at strong depolarizations). Furthermore, the decaying outward current cannot be explained by Na^+ efflux carried by electrogenic Na–Ca exchange as proposed by Mullins⁹, since it was seen in the absence of internal or external Na^+ (Fig. 2a), or in the absence of external Ca^{2+} (Fig. 2d). The experiment illustrated by Fig. 2a, b went on to test the idea that the outward current is carried by K^+ ions. We found that the decaying outward current was abolished when Cs replaced K in the internal dialysate (b), as expected if K^+ ions carry current much more effectively than Cs^+ ions.

If potassium ions carry the outward current, does K^+ move through the Ca channel itself, or through another pathway such as a K-specific channel? One possibility is that K-efflux occurs through a Ca-activated K current, $I_{\text{K(Ca)}}$. This is a reasonable hypothesis since such currents have already been found in heart (for example, ref. 14), but it would require near-perfect cancellation of I_{Ca} and $I_{\text{K(Ca)}}$ at the apparent reversal potential (Fig. 1d), a result not found in systems where these currents are known to overlap (for example, ref. 15). Figure 2c–e provides direct evidence against an explanation involving $I_{\text{K(Ca)}}$. Panel c shows inward Ca current at 0 mV, and decaying outward current at +90 mV. Removal of extracellular Ca (d) abolishes the inward Ca current, and increases the background leak conductance, but leaves the decaying outward current at +90 mV virtually unchanged. This argues against $I_{\text{K(Ca)}}$ as the mechanism of the outward current, since removal of Ca_o should quench any intracellular Ca transient that occurs beneath the surface membrane despite the presence of internal Ca buffers. The ineffectiveness of external Ca removal contrasts with the immediate abolition of the time-dependent outward current by exposure to the inorganic calcium channel blocker Cd^{2+} (Fig. 2e; see also Fig. 4d–f).

Fig. 1 Voltage and time-dependence of Ca channel current. *a, b*, Total membrane current records during a series of depolarizing pulses from a holding potential of -40 mV to levels spaced 10 mV apart. Pulses separated by at least 5 s. Capacity and leak current not subtracted. Current signal was filtered at 1 kHz and sampled at 1 kHz. Cell 64B. *c, d*, Membrane current records on a faster time scale from another cell, after digital subtraction of capacity transient and leak current. Current signals were filtered at 3 kHz. Sample rate was 10 kHz initially, then 1 kHz starting 5 ms after onset of voltage pulse. Linear 'leak' conductance and capacity current were estimated as the current during a hyperpolarizing pulse from -40 to -50 mV at the end of the run. 'Leak' conductance was 0.035 μ S. The capacitive transient settled with a final time constant of 235 μ s and its area corresponded to a total cell capacitance of ~ 184 pF. Data points are not shown for the first 300 μ s after the onset of the voltage pulse while the membrane potential settled and the total current signal came into the range of the analog-to-digital converter. Cell 79G.

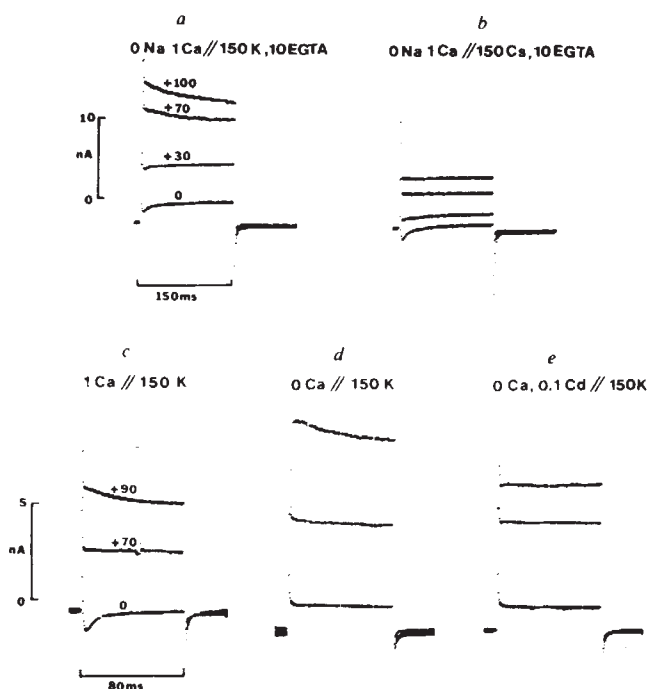
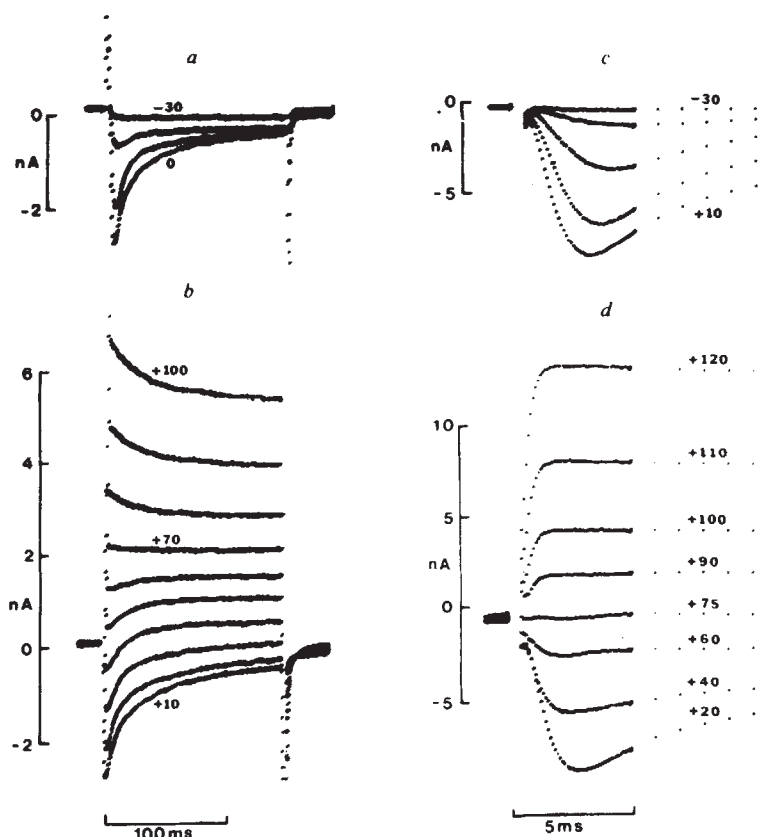


Fig. 2 Effects of removing internal K^+ or external Ca^{2+} . *a, b*: Effect of Cs^+ substitution for K^+ on decaying outward current. Total membrane currents recorded in 0 -Na 0 -K solution containing (in mM): 1 $CaCl_2$, 2.8 $MgCl_2$, 84 Cs -aspartate, 146 sucrose and 5.5 glucose, buffered to pH 7.4 with Tris. All internal solutions contain 10 mM K -EGTA. Addition of EGTA reduced the free Ca^{2+} concentration in the dialysate (kindly measured by Dr C. O. Lee using Ca^{2+} -sensitive microelectrodes) from ~ 0.4 μ M to an undetectably low level. Voltage steps from a holding potential of -40 mV to levels indicated. *b*, Records taken with 150 Cs^+ in place of K^+ in internal solution; *a*, records taken 2 min after changing internal solution to the usual 150 K^+ dialysate. Cell 66B. *c-e*, Effects of changes in external divalent cation concentration from another experiment using solutions described in text: *c*, with 1 mM Ca outside; *d*, 0 - Ca outside; *e*, 0 - Ca + 100 μ M Cd outside. Results in *d* taken after 90 s in 0 - Ca solution. Cell 47B.

Can the decaying outward current be explained by the behaviour of a conventional voltage-dependent K channel? We found no reduction of the decaying outward current with 20 mM internal tetraethylammonium (TEA) or 1 mM external 4-aminopyridine (4-AP), levels that are usually effective at inhibiting K channels in heart and other excitable cells^{16,17}. Unfortunately, higher doses of these agents were precluded by apparently nonspecific side-effects; 5 mM 4-AP produced a large outward holding current and increased leak conductance; even at 20 mM, intracellular TEA quickly diminished the inward Ca current and hastened the disappearance of all time-dependent current changes.

The most critical tests of whether the decaying outward current is carried by Ca channels came from experiments where Ca channels were blocked pharmacologically or inactivated by membrane depolarization. The idea behind the experiments was simple: if there is a genuine reversal potential where Ca channel current is zero because Ca influx and K efflux cancel, interventions that selectively inhibit Ca channels should have no effect at that potential. Figure 3 shows the effect of D600, a potent methoxy derivative of verapamil that is widely used as an inhibitor of Ca channels. A run before exposure to the drug shows a typical inversion of time-dependent current (a). After exposure to 0.3 μ M D600 (b), both decaying inward and decaying outward currents were inhibited; the residual current in D600 is nearly linear and time-independent over the voltage range illustrated. Figure 3c superimposes current traces taken at three key test levels before and after D600. At the putative reversal potential, where time-dependent current vanishes, control and D600 traces coincide, just as expected if the driving force for net Ca channel current were zero and if D600 had no effect on leakage current.

The voltage-dependence of peak current and late current as obtained by this D600 dissection are plotted in Fig. 3d. Some features of the current-voltage relationships are surprising. First, late D600-sensitive current is appreciable, even though the predominant time-dependent current change seems largely complete at the end of the 150 -ms clamp pulse (c). Second, persistent D600-sensitive current is significant even over the range between -50 and -30 mV, where there is very little

time-dependent inward current. Third, peak and late D600-sensitive currents grow quite large at strongly positive potentials; both I - V relationships show upward curvature between +30 and +100 mV.

The results illustrated in Fig. 3 are representative of a total of five experiments with 0.1–5 μ M D600. The simplest interpretation is that Ca channel current truly reverses and that D600 blocks current flow in either direction. It could be argued that the null effect at E_{rev} is fortuitous, and that D600 not only blocks the Ca channel but also a voltage-dependent K channel. However, this explanation is very unlikely in view of the more extensive evidence illustrated in Fig. 4. Using the approach introduced in Fig. 3, we looked at varying degrees of Ca channel inhibition, not only by D600 (Fig. 4a–c), but also by the inorganic blocker Cd^{2+} (Fig. 4d–f), and by inactivation with depolarizing prepulses (Fig. 4g–i). In all three types of experiments, partial reduction of inward current below E_{rev} was associated with a partial decrease of outward current above E_{rev} , while the nearly time-independent current near E_{rev} remained essentially unchanged. These results provide strong evidence for a genuine reversal of Ca channel current at strong depolarizations. At the nearly physiological concentrations of Ca^{2+} and K^{+} used in these experiments, the outward current is due to K^{+} efflux through the channel, as originally hypothesized⁸. K^{+} ions are not the only monovalent cations which can permeate the Ca channel. An outward movement of Cs^{+} ions can also be detected in external media lacking permeant divalent cations or containing barium instead of calcium. Like the outward K^{+} movement, the outward Cs^{+} current can be inhibited by D600, Cd, or depolarizing prepulses; these interventions have no effect

at the reversal potential seen with Ba^{2+} outside and Cs^{+} inside, but reduce inward and outward currents recorded on either side of E_{rev} ¹⁸.

There is no conflict between these results in heart cells and earlier studies in dialysed molluscan nerve cells that did not show outward current through Ca channels^{5–7}. The neurone experiments were done with Cs^{+} or $Tris^{+}$ as the intracellular cations, with Ca^{2+} outside. In similar ionic conditions, our heart

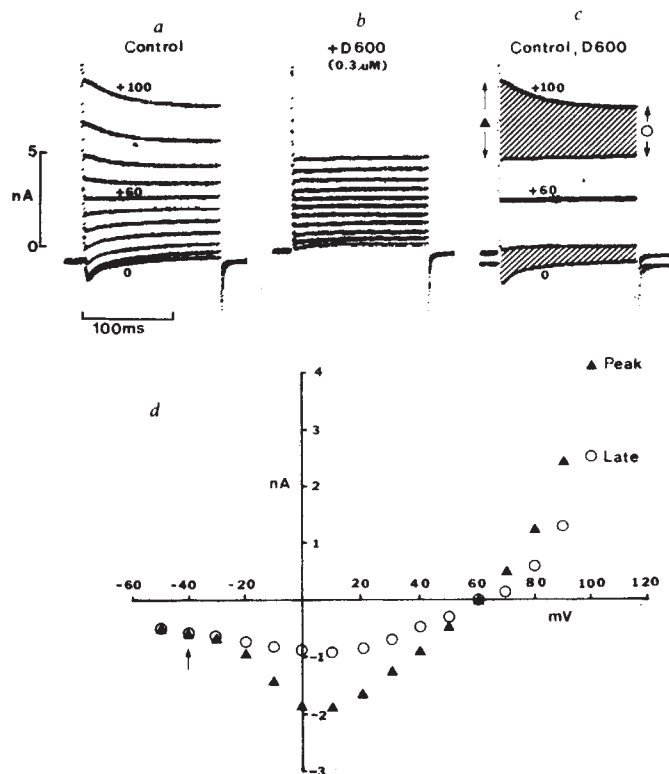


Fig. 3 Pharmacological dissection of calcium channel current using D600. *a*, Total membrane currents recorded during depolarizing pulses from -40 mV to levels spaced 10 mV apart, in conditions similar to Fig. 1b. *b*, Current records associated with the same set of voltage pulses after 5 min exposure to 0.3 μ M D600. *c*, Repeat of current traces in *a* and *b*, for pulses to 0, +60 and +100 mV. The shaded areas indicate D600-sensitive current. Peak ($\blacktriangle$) and late ($\circ$) D600-sensitive current at 100 mV were measured as indicated. *d*, Voltage-dependence of peak and late D600-sensitive current measured from signals shown in *a* and *b* and from records during pulses to levels below 0 mV. Arrow marks holding potential. Symbols at -40 and -50 mV denote changes in steady current. Cell 64A.

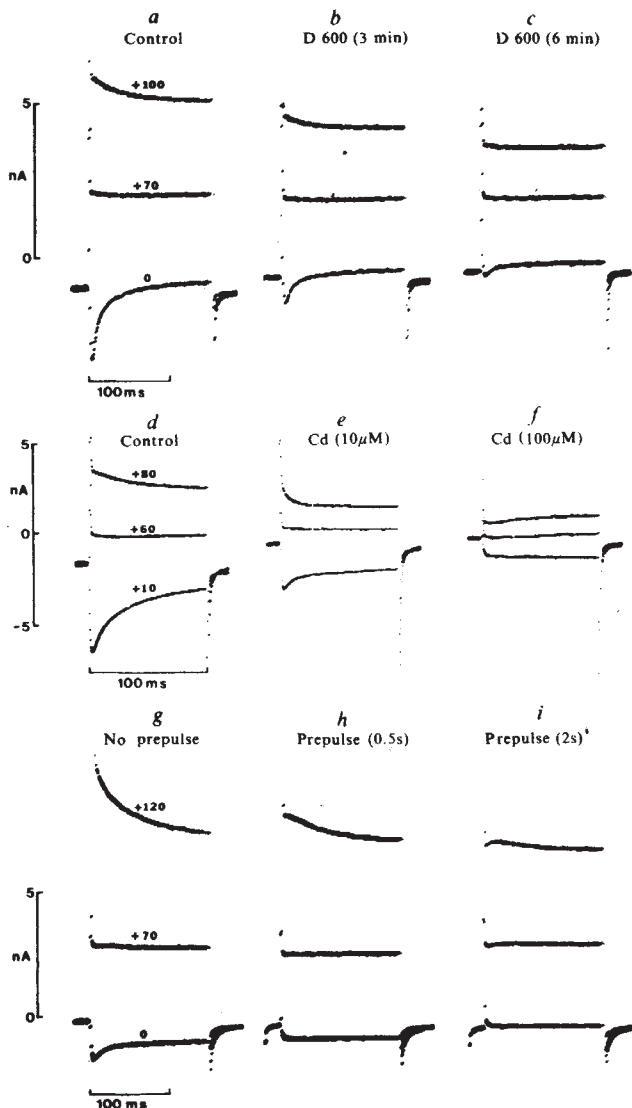


Fig. 4 Graded effects of interventions that reduce Ca channel conductance. *a*–*c*, Progressive development of D600 inhibition: *a*, 1 min after beginning D600 exposure, before any significant Ca channel block; *b*, after 3 min, when inward Ca current was roughly halved; *c*, after 6 min, when D600 effect had reached steady state. Cell 65C. Panels *d*–*f*, graded effect of external Cd^{2+} . The records in *d* and *e* were taken within a few minutes after increasing the external Cd^{2+} concentration, after block had reached steady state. In a total of six experiments using 10–500 μ M Cd^{2+} , four cells showed no significant change in the level of current at the apparent reversal, one cell showed an upward shift, and one cell gave a downward shift. The Cd^{2+} -sensitive current resembled the D600-sensitive current illustrated in Fig. 3d. The speeding of inactivation evident in panel *e* was a consistent finding at low levels of Cd^{2+} . Cell 46A. Panels *g*–*i*, graded inactivation by conditioning prepulses to -10 mV. The current records were evoked by test pulses from a holding potential of -40 mV to the levels indicated. *g*, No prepulse preceding test pulses; *h*, 0.5 s conditioning prepulse, separated from test pulses by 20 ms repolarization to -40 mV; *i*, 2 s conditioning prepulse. Time-dependent current were reduced to a greater extent in *i* than in *h*; the Ca current recorded during prepulses to -10 mV showed a corresponding slow decline between 0.5 and 2 s. Cell 73A.

cells also lack decaying outward currents over the voltage range between 0 and +120 mV (Fig. 2*b*; ref. 18). It is interesting that outward Cs current can only be detected in the absence of external Ca. Presumably, Ca^{2+} ions bind to the channel much more strongly than Ba^{2+} ions¹, preventing significant outward movement of Cs^+ , but not the efflux of the more permeant K^+ .

These experiments show for the first time the feasibility of studying cardiac Ca channels while changing intracellular ions at will. As in the case of various patch clamp techniques^{19,20}, the suction pipette method offers its own unique combination of advantages. The relatively large pipettes used here give direct recordings of ensemble properties of ionic channels, like whole cell recordings using fine-tipped pipettes^{19,21}, but they also allow manipulation of the medium on the cytoplasmic side of the membrane, like cell-free patches^{19,20}. Lability of Ca channel function does not seem to be a serious problem: inward and

outward Ca channel currents often remain relatively stable for up to 30–40 min with external Ca and for even longer with external Ba. Systematic variations in intracellular constituents might give further improvements. But our experience already augurs well for future studies on modulation of Ca channels by adrenaline, acetylcholine or digitalis, and the role of putative intracellular mediators such as cyclic nucleotides or Ca^{2+} ions^{2,3,22}. Intracellular Ca is also thought to promote Ca channel inactivation in a variety of excitable cells²³ including certain heart cells (for example, ref. 24). However, this cannot be the only mechanism since, as Fig. 2*d* shows, outward current through these Ca channels undergoes inactivation even in the absence of permeant divalent cations.

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Properties of single calcium channels in cardiac cell culture

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The permeability of cell membranes to Ca^{2+} ions is of great importance for a variety of cellular functions such as secretion of neurotransmitters and hormones, or contractile activation of heart cells. In several cell types including cardiac muscle, Ca^{2+} channels are gated by membrane potential and regulated by neurotransmitters^{1–3}. We have used the patch clamp technique⁴ in order to obtain information about the properties of single Ca channels in cultured heart cells. With Ba^{2+} carrying the inward current the single channel slope conductance is about 25 pS, and the probability of channel opening increases markedly with voltage. The channels become inactivated over a membrane potential range of about 60 mV. The mean channel open time is about 1 ms at 25 °C. However, a second much slower clustering behaviour primarily determines the slow activation time course of averaged currents. Isoprenaline does not affect single channel conductance, but seems to lengthen the mean open time and to decrease intervals between bursts.

Tissue cultured myocytes were obtained from neonatal rat hearts^{5,6}. After seeding, the cells were incubated for 2–4 days in a culture medium containing 0.1 mM 8-bromo cyclic AMP to increase the likelihood of detecting single Ca channels⁷. Most cells used for our experiments had spindle-like shapes and showed striations. Single-channel currents were recorded from cell-attached membrane patches with seals between 10 and 100 GΩ (ref. 4). The recording pipette contained 96 mM BaCl_2 solution with 20 μM tetrodotoxin (buffered at pH 7.4 with 10 mM HEPES); thus, the current carrier was Ba^{2+} rather than

Ca^{2+} . The composition of the bathing solution was (mM): NaCl 137, KCl 5.4, MgCl_2 1, CaCl_2 0.02, glucose 10, HEPES buffer 10 (pH 7.4); temperature 24–26 °C. Consistent results have been obtained from experiments on 24 patches with Ca channel activity, most of which had only a single Ca channel present. Our analysis of single Ca channel properties presented here is based primarily on results obtained from five patches, each of which provided rather complete information; three of these had only a single Ca channel present.

Calcium channels switch randomly between open and closed states, and appear not to dwell at intermediate conductance levels; some depolarizations produce one or more channel openings of various durations, whereas for others, the channel fails to open at all (Fig. 1).

The steady-state probability that a channel will be open depends on membrane potential, as does the current that flows while the channel is open (Fig. 2). We have estimated the single channel current (*i*) by eye (Fig. 2*a*) and calculated open probability (*p*) from

$$I = Npi \quad (1)$$

where the mean current *I* was found by time-averaging the current flowing through calcium channels over 50 or 100 ms, and *N* is the number of channels functioning in the patch (*N* = 1 for Fig. 2). The probability of a channel being open increases with depolarization, at a maximum rate of about *e*-fold per 10 mV, from nearly zero at a membrane potential of –30 mV to 0.7 at around +30 mV. Over this same voltage range, the open channel current–voltage relationship is approximately ohmic with a slope conductance of about 25 pS (isotonic BaCl_2 extracellularly).

During long depolarizing steps Ca channels show inactivation that is 20 or 30 times slower than activation (Fig. 3*a*). We studied steady-state inactivation by measuring Ca channel currents at a fixed potential after holding the membrane potential at various conditioning levels (Fig. 3*b*). This inactivation occurs through a decrease in the probability that a channel is available to open, and not through a decrease in single channel current, *i*. Steady-state inactivation increases with conditioning depolarization and can be fitted by a Boltzmann distribution. Channels are half-inactivated at a membrane potential of about

-35 mV, while channels just begin to be activated with depolarizations slightly greater than this (about -30 mV).

We have estimated the average duration of a channel opening, $\bar{t}$, by the equation

$$\bar{t} = \frac{p}{r} \quad (2)$$

where p is the fraction of the time a channel spends open (from equation (1)) and r is the average opening rate obtained by counting the number of opening transitions n during the period T over which the time-averaged current was calculated ($r = n/T$). When necessary the average opening rate was corrected for openings too brief to be detected; this correction assumed an exponential distribution of open times, and was at most 1%. The mean open time was always close to 1 ms. For example, for the experiment that yielded the records shown in Fig. 2,

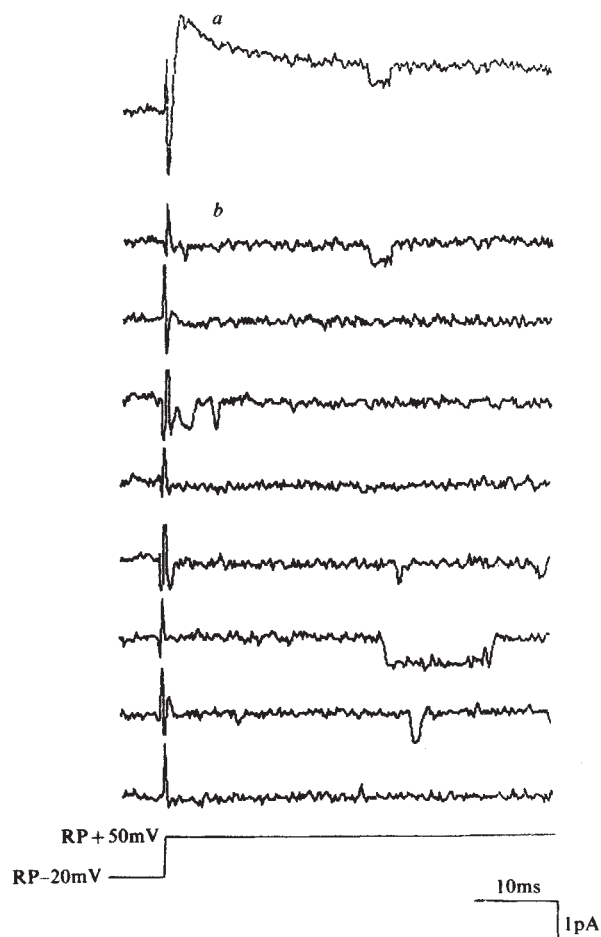


Fig. 1 Single Ca channel currents from cultured rat heart cells. In record *a*, the capacitive transient was reduced by analog compensation⁴. The eight sequential records in *b* have had the remaining capacitive transient and the leakage current subtracted (the first record in *b* is derived from *a*). Records were inspected visually, and portions containing channel activity or obvious artefacts were excluded from the leakage current average. Membrane potential of the patch was held at 20 mV negative to the resting potential (RP) and stepped to RP + 50 mV. Resting potential of this cell was estimated to be -50 mV; this was done by comparing unit currents through Ca-activated inward current channels⁵ in these cell-attached patches with the unit current carried by Ba²⁺ ions through such channels in detached patches, for which the true membrane potential is known (D. Colquhoun, E. Neher, H.R. and C.F.S., unpublished results). These channels were frequently observed in these experiments, but in the voltage range where we have studied Ca channel current they carry only a small unit current; thus, they can easily be distinguished from the Ca channel currents and make no appreciable contribution to the observed current.

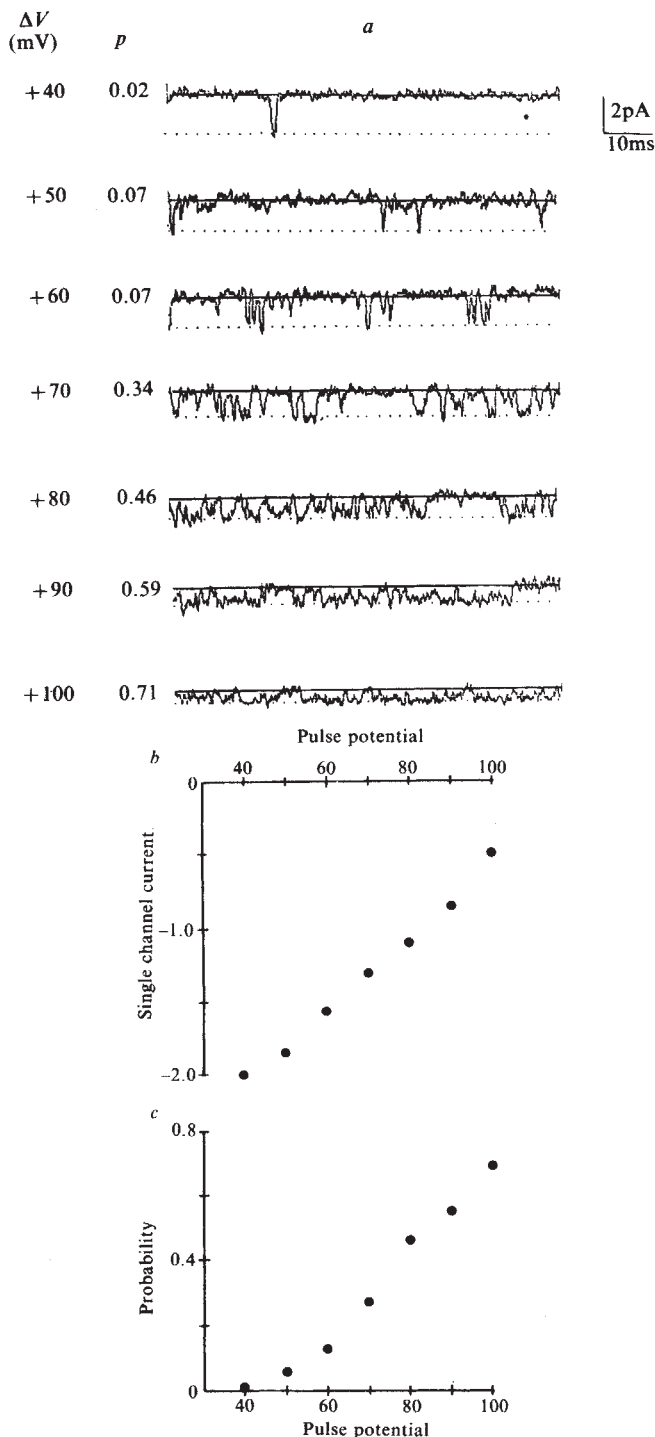


Fig. 2 Voltage dependence of single Ca channel currents. *a*, Ca channel currents at several voltages. Membrane potential was stepped by the indicated step size (ΔV) from a holding potential of RP - 10 mV (RP $\approx$ -60 mV). Leakage current has been subtracted from these records. The solid lines indicate the baseline after leak subtraction. The dotted lines indicate the measured open-channel current. Beside each record is the average probability (p) that the channel was open, as determined from the individual record shown (see text, equation (1)). This patch had only a single active Ca channel. Data were filtered at 1 kHz. *b*, Voltage dependence of open channel current. Open channel current was fitted visually for the experiment in *a* for each membrane potential. A pulse potential of 40 mV corresponds approximately to a membrane potential of -30 mV, as above. *c*, Voltage dependence of Ca channel gating. The time-averaged probability that the single active Ca channel in this patch was open was determined (for each voltage) by dividing the average current by the open channel current (equation (1)). Average current was measured over a 90 ms interval beginning 5 ms after the start of each pulse.

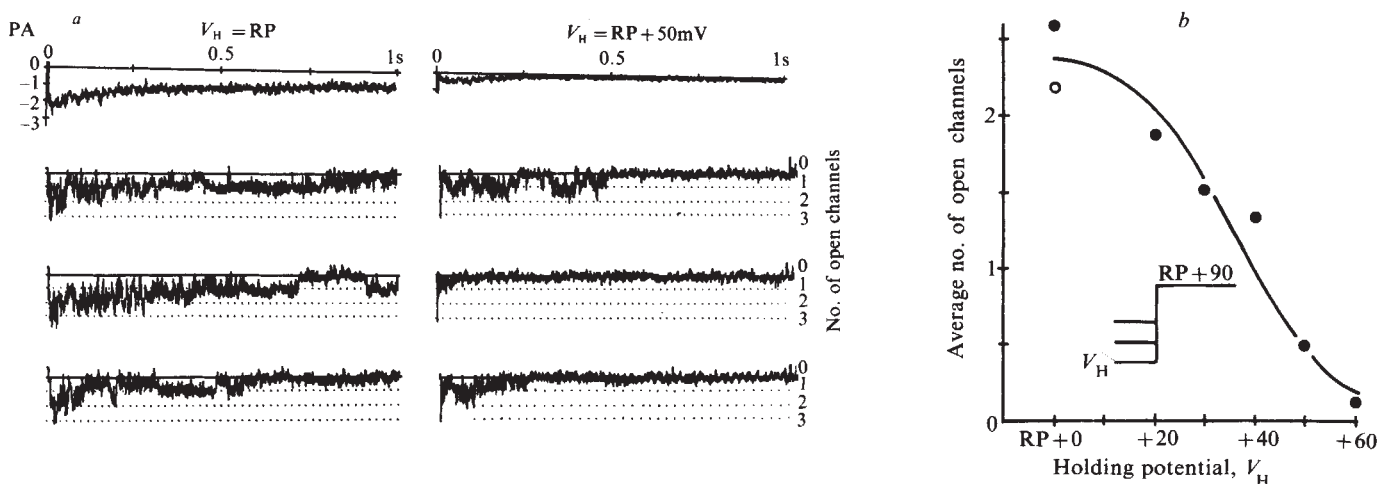


Fig. 3 Inactivation of Ca channel currents. *a*, Ca channel currents at a fixed potential, stepping from two different holding potentials. Left, V_H (holding potential) = RP; right, $V_H = RP + 50mV$ (RP $\approx -70mV$). Command potential is RP + 90 mV in both cases. At top is the average current, calculated from about 20 individual records, for each holding potential (leakage current subtracted). There is no significant change in time-to-peak when the averaged records are scaled to the same amplitude. Below are examples of three individual records from each holding potential (same scale as averages). Dotted lines on the individual records are spaced 0.86 pA apart, which is the size of a single channel current at RP + 90 mV. Notice the additional clustering which occurs on a very slow (hundreds of milliseconds) time scale. This patch showed a maximum of four open channels for large depolarizations. Data were filtered at 1 kHz. *b*, Voltage dependence of Ca channel inactivation. The time-averaged number of open channels in the first 50 ms of a test pulse to RP + 90 mV is plotted as a function of holding potential. The average number of open channels is found by dividing the average current during the first 50 ms of the test pulse by the unit open channel current which did not vary with holding potential. The solid line is a Boltzmann distribution:

$$N = N_0 [1 + \exp((V_m - V_{0.5})/k)]^{-1}$$

with parameters chosen for the best visual fit: $N_0 = 2.4$ open channels; $V_{0.5} = RP + 35mV$; $k = 10mV$. The open circle is a repeat of the $V_H = RP$ point, done at the end of the inactivation series. Observations of Ca channel activation before and after the inactivation experiment illustrated here agree closely with the activation curve in Fig. 2*b*.

the mean open time increased from 0.65 ms at a pulse potential of 60 mV to 1.4 ms for an 80 mV pulse. Although the standard errors of these measurements are less than 10% of the mean, we estimate that systematic errors of closer to 15% are present due to uncertainties in counting opening transitions (the interested reader should count openings in the records of Fig. 2). Our values for mean open time are therefore close to the estimates reported for Ca channels in snail neurones⁸ and bovine chromaffin cells⁹.

The Ca channels studied here exhibit distinctive gating behaviour. Their openings are not dispersed at random throughout the period of depolarization, but rather tend to occur in groups of two or more closely spaced openings separated by longer silent times (see Fig. 2*a*, +60 and +70 mV). It is as if the channel enters an 'activated' condition in which it rapidly flickers open and closed. The average current is activated (see Fig. 3*a*, upper row) with a time constant on the order of 10 ms, and this longer time seems to reflect the burst length rather than the characteristic time (~ 1 ms) of the individual openings.

The β -adrenergic agonist isoprenaline, mimicking the action of the hormone adrenaline, causes an increase in cardiac calcium currents^{1,2,10}. We have investigated the effects of this drug at the single channel level in several preliminary experiments. Isoprenaline caused no increase in the single channel current, but in one experiment (10 μM) it produced a 60% increase in the mean open time which was manifest as a corresponding increase in the probability from 0.06 to 0.09 ($V_m \approx +10mV$). A similar but smaller effect was observed in a second experiment (1 μM). If this effect is confirmed by a more complete analysis, isoprenaline can be viewed as causing an increased conductance¹⁰, at least partly, by altering the fraction of time an 'activated' channel spends in its open state (see ref. 2).

The kinetic properties of single Ca channel currents qualitatively agree with previously reported kinetic data on global Ca currents in a variety of cardiac tissues¹. We find a steep increase in the probability of channels opening over the same potential range where activation of Ca conductance occurs^{1,11}. Our single channel records exhibit steady-state inactivation kinetics similar to those reported for global Ca currents^{1,11}. However, in con-

trast to previous results there is a smaller range of overlap between potentials where channels open and become inactivated. This may partly be due to the fact that we have used isotonic BaCl₂ solution in our pipette which is known to alter surface charges¹² and thereby shift activation and inactivation ranges along the voltage axis. We have used Ba²⁺ instead of Ca²⁺ ions because of their higher permeability through Ca channels^{3,8}.

Our measurements of single channel conductance yield values that are more than an order of magnitude larger than those estimated from fluctuation analysis in molluscan neurones in comparable conditions¹³. However, they are consistent with two reports on single Ca channels in snail neurones⁸, and bovine chromaffin cells⁹. In the latter note, bursts of channel openings, presumably similar to those observed in our study, have also been mentioned.

In comparison with Na channels^{14,15}, Ca channels show a much more complex kinetic behaviour. There is a fast gating process (mean channel open time ~ 1 ms at 25 °C) and a slower one that results in bursts. It is interesting to speculate that one of the gating processes is regulated by some intermediate metabolic step, such as phosphorylation of the Ca channel. In this respect it will be important to pursue our preliminary isoprenaline results, since β -adrenergic catecholamines are thought to regulate the Ca conductance through such a mechanism^{1,2}.

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Calcium-binding protein parvalbumin is associated with fast contracting muscle fibres

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The basic mechanism underlying the contraction–relaxation cycle of vertebrate muscles is a Ca^{2+} exchange between the sarcoplasmic reticulum and the myofibrils. Relaxation is achieved by retrieval of Ca^{2+} from the myofibrils and transfer to the sarcoplasmic reticulum. However, it is uncertain whether the rate of Ca^{2+} uptake by the sarcoplasmic reticulum can account for the known speed of relaxation^{1,2}. In fast contracting muscles the Ca^{2+} -binding protein parvalbumin is postulated to facilitate relaxation^{3–8}. Using immunohistochemical techniques, we show here that parvalbumin is located exclusively in type II (fast-twitch) mammalian skeletal muscle fibres which can be further subdivided into five subgroups displaying distinct staining intensities. As the active state decays more rapidly in fast than in slow muscles^{9,10}, our results support the contention that parvalbumin may be concerned with rapid muscle relaxation^{3–8} and in addition suggest a range of relaxation properties in muscle fibres belonging to the same histochemical fibre type.

Newborn and adult Wistar rats of both sexes were killed and various skeletal muscles quickly dissected out, stretched and frozen in isopentane cooled by liquid nitrogen. The muscles were then divided and the respective halves separately pro-

cessed for histochemical and immunohistochemical localization. Additionally, heart muscle tissue taken from the atria and ventricles, smooth muscle tissue of the gut, great vessels and non-pregnant uteri, as well as specialized, small cross-striated muscles with a high intrinsic speed of contraction (external eye muscles and intrinsic laryngeal muscles⁹) were dissected out, fixed in Bouin fluid and embedded in paraffin. The high solubility of parvalbumin prevented the use of unfixed or postfixed cryostat sections for the immunohistochemistry. Monospecific antisera to rat muscle parvalbumin were used in dilutions up to 1:30,000 in phosphate-buffered saline (PBS). The specificity of the antisera has been described elsewhere¹¹; there is no cross-reaction¹² with troponin-C, calmodulin or S-100 protein. Sections were further processed according to the unlabelled antibody technique of Sternberger¹³. Briefly, goat-anti-rabbit IgG (Nordic) was applied for 30 min, followed by peroxidase–antiperoxidase complex (Sternberger–Meyer Inc.). The peroxidase-dependent reaction was detected by means of diaminobenzidine HCl/ H_2O_2 . Control slides were processed identically and simultaneously with preimmune sera of the same animal and with preadsorbed antisera.

Parvalbumin immunoreactivity in the extensor digitorum longus muscle of newborn rats appears on postnatal days 3 or 4. In adult rats, anti-parvalbumin antisera stain extensor digitorum longus extrafusal muscle fibres in at least five different degrees of intensity (Fig. 1a). In the soleus muscle the parvalbumin immunostaining is much less conspicuous (Fig. 1b). The parvalbumin immunostaining pattern (Fig. 2a) was correlated with histochemical fibre typing by staining serial sections for myofibrillar ATPase^{14,15} after preincubation at pH 4.3 (Fig. 2b) and with formalin followed by pH 10.6 (Fig. 2c). Several laboratories have shown near-equivalence of formalin-based and acid-based subdivisions of fast fibres^{16–18}. Accordingly, formaldehyde-resistant fast fibres will here be designated type IIA, and formaldehyde-sensitive ones type IIB. The muscle fibres could thus be histochemically classified into the main groups: type I (Fig. 2b) (slow-twitch, oxidative), type IIA (fast-twitch, oxidative-glycolytic) and type IIB (fast-twitch, glycolytic)^{14,15,19} (Fig. 2c). The type IIB fibres showed the strongest immunoreactivity towards parvalbumin antisera (Fig. 2a) and could be differentiated into two subgroups showing graded

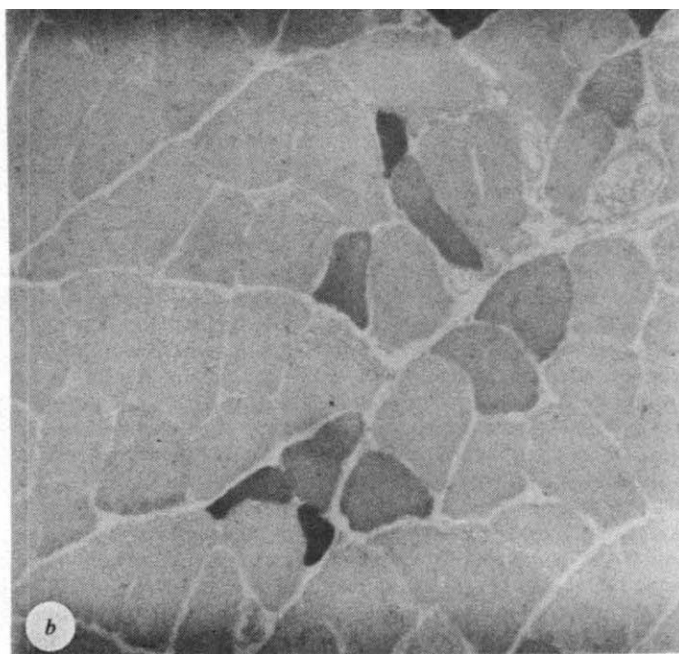
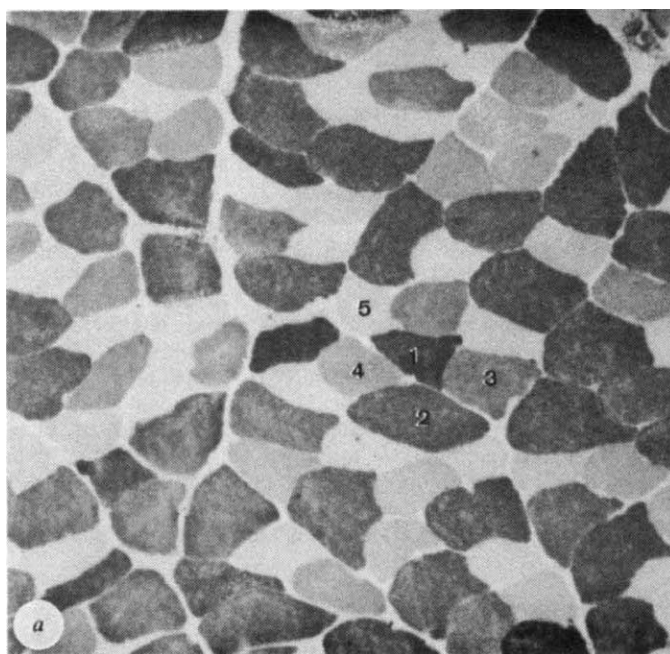


Fig. 1 *a*, Survey of a transverse section through the belly of the musculus extensor digitorum longus (EDL; fast contracting/fast relaxing⁹) of an adult rat incubated with parvalbumin antiserum and processed by the peroxidase–antiperoxidase technique¹³. The parvalbumin immunostaining shows a chequered appearance with at least five different shadowings in staining intensity, even at the extreme antiserum dilution of 1:20,000. *b* Shows a cross-section of the ipsilateral soleus (slow contracting/slow relaxing⁹) of the same animal processed identically. In the soleus very few muscle fibres are labelled by the parvalbumin antiserum. $\times 150$.

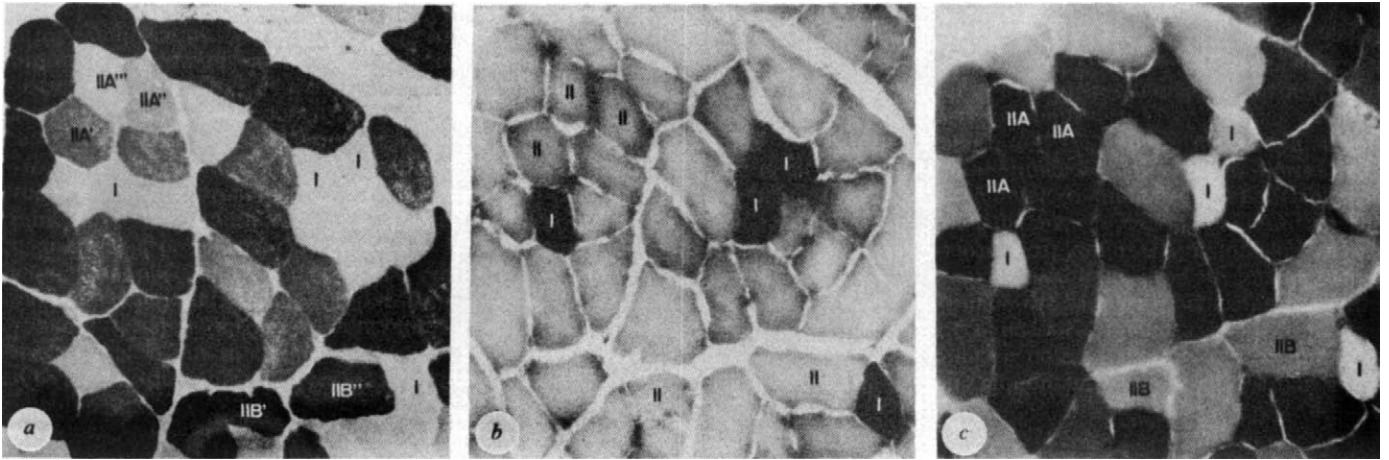


Fig. 2 Consecutive transverse sections of the EDL of an adult rat, analysed for parvalbumin immunoreactivity (a) and enzyme-histochemical fibre types (b, c). Adult Wistar rats (Ivanovas) were killed by a blow to the head and the muscles quickly dissected out, frozen in isopentane cooled by liquid nitrogen and halved with a precooled razor blade. One part was freeze-substituted with acetone for 1 week at -70°C and fixed at the same temperature with a mixture of acetone (90 ml), formalin (40% per 5 ml), acetic acid (1 ml) and water (4 ml) for another week. The specimens were thawed, embedded in Paraplast and the sections incubated with antisera against rat muscle parvalbumin in dilutions varying between 1:5,000 and 1:30,000 for 48 h in a moist chamber at 4°C . The immune complex was visualized by the unlabelled antibody technique of Sternberger¹³. Identical distribution and localization of parvalbumin immunoreactivity were achieved after perfusion fixation of the rat's body with Bouin fluid and embedding of the muscles in paraffin (see Fig. 4 legend). The second half of the muscle was cut in a cryostat and the sections processed for acid-stable (pH 4.3) ATPase activity (b) and formalin/alkali-stable (pH 10.6) ATPase activity (c), following established procedures¹⁴⁻¹⁸. Corresponding areas were photographed and muscle fibres identified. Type IIB fibres (b, c) display two distinct degrees of staining intensity with parvalbumin antisera (IIB', IIB'' in a) while type IIA fibres (b, c) may even show three (IIA', IIA'', IIA''' in a). IIA''' fibres are actually devoid of parvalbumin immunoreactivity and in this respect resemble type I fibres. Note that the strongest immunolabelling is detectable in the small-calibre IIB' fibres (a). $\times 148$.

differences in staining intensity. The type IIA muscle fibres were moderately stained in three distinct degrees of intensity (Fig. 2a). Type I muscle fibres always remained nonreactive (Fig. 2a). In muscles spindles, one nuclear chain fibre was immunolabelled, whereas bag fibres were not (Fig. 3). High parvalbumin immunoreactivity was detectable in fibres belonging to the global portion of the musculus rectus lateralis oculi (Fig. 4b) and in the musculus thyroarytenoideus lateralis (Fig. 4a). The slow tonic fibres in the orbital layer of external eye muscles (Fig. 4b) as well as the musculus thyroarytenoideus medialis (vocalis) were nonreactive (Fig. 4a). Cardiac muscle fibres and smooth muscle cells were never labelled by parvalbumin antisera (not shown).

The immunostaining was reproducible for a wide range of antiserum dilutions. Identical correlations with histochemical fibre typing were obtained using other fixatives and embedding procedures (see figure legends). The staining pattern was homogeneous and evenly distributed over the whole muscle fibre in longitudinal section (not shown) and in cross-section.

A discussion of the role of parvalbumin in muscle hinges on the hypothesis that parvalbumin facilitates the relaxation by shuttling Ca^{2+} from troponin-C to the sarcoplasmic reticulum³⁻⁸. The sarcoplasmic reticulum is actually well developed in type II muscle fibres²⁰, which are rich in parvalbumin, and type II muscle fibres form the bulk of fast muscles, which as a whole are indeed fast relaxing. Our observation of a high parvalbumin content in such fibres supports the notion that parvalbumin is implicated in muscle relaxation, as the decay of the active state is shorter in fast than slow motor units. This assumption is strengthened by the observation that parvalbumin immunoreactivity appears on days 3 and 4 after birth in the extensor digitorum longus, coincident with changes in contraction properties, in particular the decrease in the relaxation time after a twitch or tetanus^{21,22}. Similarly, it is probable that the parvalbumin-positive intrafusal nuclear chain fibre relaxes faster than do nuclear bag fibres²³.

The teleological meaning of rapid relaxation is that muscle fibres are sooner ready for a new contraction. It is therefore not surprising that parvalbumin immunoreactivity is particularly high in fibres of muscles characterized by very high contraction frequencies. In the rat^{9,10} these muscles are the external eye muscles and the inner laryngeal muscles, both displaying the highest intrinsic speed of shortening and the shortest twitch half-relaxation times.

Type I muscle fibres show a relatively poorly-developed sarcoplasmic reticulum and lack parvalbumin immunoreactivity. Like cardiac muscle, they are well provided with mitochondria which may be involved in Ca^{2+} homeostasis²⁴. As no isoforms of parvalbumin have been found in rat skeletal muscles²⁵, the relative staining intensities presumably correspond to differences in the concentration of parvalbumin within these muscle fibres and may reflect a continuum in relaxation properties. This is consistent with the finding of metabolic subpopulations of type II fibres and the proposition of a dynamic continuum of muscle fibres²⁶⁻³¹ correlated with the wide spectrum of contraction times^{32,33}.

An incidental effect attributed to parvalbumin is that it might, by releasing heat as it takes up Ca^{2+} , contribute substantially to the 'unexplained' heat output displayed by many muscles early in tetanus³⁴. This hypothesis may now be tested by comparing muscles with high and low parvalbumin concentrations.

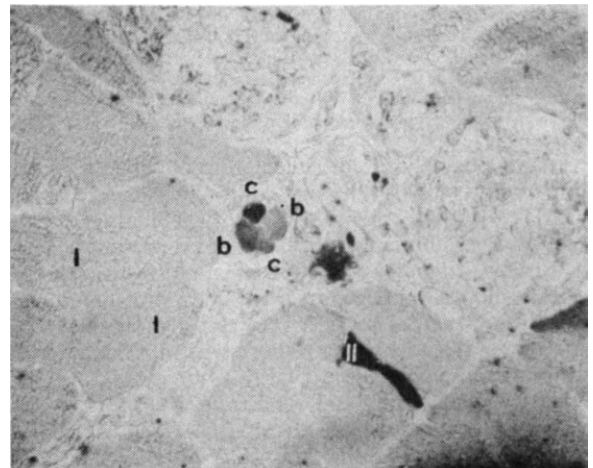


Fig. 3 Cross-section through the intracapsular polar region of a muscle spindle of the soleus muscle. Four miniature muscle fibres are suspended in the spindle capsule. One chain fibre (c) is strongly parvalbumin-reactive whereas the second chain fibre (c) as well as the bag fibres (b) are only weakly labelled. The parvalbumin immunostaining of soleus muscle spindles was always unequivocal, in contrast to that of identically treated EDL muscle spindles. I and II: types I and II extrafusal muscle fibres (as determined in following sections treated for the histochemical detection of ATPase). $\times 250$.

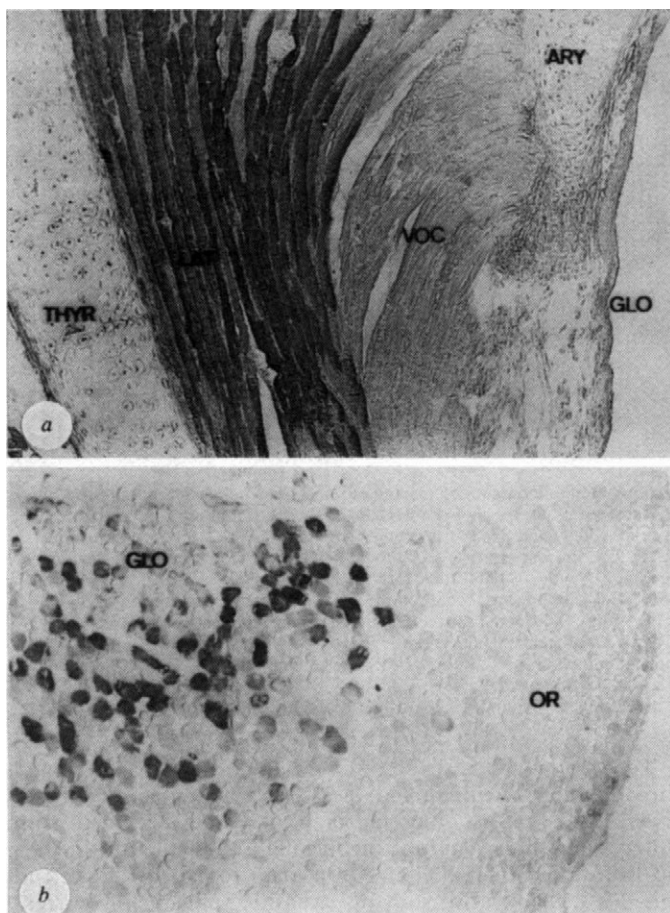


Fig. 4 Adult Wistar rats were perfusion-fixed with Bouin fluid and the specimens dissected out and embedded in paraffin. Sections 5 μ m thick were mounted on chrome-alum gelatine-coated slides, dewaxed and incubated with primary and secondary antisera. *a*, Cross-section of the rat larynx at the level of the vocal ligament. Parvalbumin-immunoreactive fibres are restricted to the thyroarytenoideus lateralis muscle (LAT) and absent from the thyroarytenoideus medialis (vocalis, VOC). The LAT has extremely short contraction and half-relaxation times^{9,40}, a well developed sarcotubular system^{40,41} and histochemically a very homogeneous IIB fibre type population, whereas the thyroarytenoideus medialis is histochemically heterogeneous (not shown). Almost all muscle fibres in the LAT show a comparable staining intensity with parvalbumin antisera over a wide range of antiserum dilutions. ARY, cartilago arytenoidea, THYR, cartilago thyroidea, GLO, glottis. $\times 125$. *b*, Cross-section of an external eye muscle. Only fibres restricted to the global portion (GLO) of the rectus lateralis muscle are recognized by parvalbumin antisera, whereas the small-calibre fibres of the orbital layer (OR), with a known slower contraction time⁴², remain unlabelled. The intensity of immunostaining with parvalbumin antisera varies among muscle fibres, reflecting the presence of a wide range of different histochemical fibre types⁴³. $\times 100$.

Our results demonstrate an association of the Ca^{2+} -binding protein parvalbumin with the fast-contracting muscle fibres of rat muscles, in agreement with earlier findings on fish muscles³⁵⁻³⁹. Parvalbumin could therefore represent a reliable system for Ca^{2+} homeostasis, allowing rapid cyclic relaxation in phasically active muscle fibres. Furthermore, parvalbumin immunohistochemistry seems to be a very sensitive auxiliary technique to enzyme histochemistry and myosin immunohistochemistry for classifying muscle fibres into various subtypes.

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Genetic regulation of resistance to intracellular pathogens

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Natural resistance of mice to infections with *Salmonella typhimurium* and *Leishmania donovani* is regulated by chromosome 1 gene(s) designated *Ity* and *Lsh*, respectively^{1,2}. Given the fact that these two microorganisms are taxonomically and antigenically distinct, and yet the host response to them is regulated by the same locus or complex^{3,4}, one might expect that the resistance to other intracellular pathogens would be controlled similarly. Innate resistance of inbred mice to infection with *Mycobacterium bovis* (BCG) is regulated by a single, dominant, autosomal gene designated *Bcg* which is known to exist in two allelic forms: resistant *Bcg*^r and susceptible *Bcg*^s (ref. 5). The distribution of *Bcg*^r and *Bcg*^s alleles, among a total of 14 inbred and 38 recombinant inbred (BXD and BXH) strains, matches exactly that established for resistant and susceptible alleles of *Lsh* (gene controlling resistance to *Leishmania donovani*) and *Ity* (gene controlling resistance to *Salmonella typhimurium*), suggesting that resistance to all these pathogens is controlled by the same chromosome 1 locus. The existence of such a chromosomal locus is further supported by the *Bcg*-*Lsh* and *Bcg*-*Ity* linkage as established by formal backcross analysis.

Mice of selected inbred and recombinant inbred strains were infected intravenously with a dispersed inoculum of BCG⁵ at a dose of $2-3 \times 10^4$ colony-forming units (CFU) of BCG. The number of viable BCG organisms in the spleen was determined 3 weeks after infection. All the inbred mouse strains which were tested segregated into two distinct groups with respect to the host response to BCG infection: resistant strains (*Bcg*^r), in which practically no proliferation of BCG inoculum occurred, and susceptible strains (*Bcg*^s), which allowed up to 100-fold BCG multiplication in their spleens (Table 1). Segregation into resistant and susceptible strains matches exactly that which was established for *Ity*- and *Lsh*-regulated resistance to *S. typhimurium* and *L. donovani*^{7,8}.

Recombinant inbred strains BXD and BXH (originating from the BCG-susceptible C57BL/6J progenitor, and the BCG-resistant DBA/2J and C3H/HeJ progenitors, respectively), when infected with BCG, also segregated into two distinct groups (Figs 1, 2). The strain distribution pattern (SDP) of *Bcg*^r and *Bcg*^s alleles among BXD and BXH strains was found to be completely concordant with that established for *Ity*^r, *Ity*^s and *Lsh*^r, *Lsh*^s alleles^{3,4}. One strain deserves a comment: BXH-2 typed as susceptible to BCG while reported to be *Lsh*^r and *Ity*^r. However, the (C57BL/6J × BXH-2)_{F1} hybrid mice were found to be resistant to BCG ($\log_{10}$ BCG = 4.69 ± 0.14), thus clearly indicating that the phenotypic susceptibility to BCG of BXH-2 strain is caused by a factor unrelated to the *Bcg* gene that overrides the resistance conferred by the *Bcg*^r allele. It is of interest that the BXH-2 strain was also found to express high levels of murine leukaemia virus, and has a high incidence of lymphoma. The basis for these characteristics is not fully understood, but was acquired after the strain was already inbred⁹.

We have also examined the linkage between *Lsh* and *Bcg* in a backcross population. *F1* hybrids between B10.A (*Bcg*^s *Lsh*^s) and A/J (*Bcg*^r *Lsh*^r) were mated to the susceptible B10.A parent to produce backcross progeny. These were infected with BCG, and splenectomized 3 weeks later for *Bcg* typing. The animals were then infected with *L. donovani* after a further 3 weeks and the *Lsh* type was established in their livers⁸. Preliminary experiments confirmed that neither splenectomy

Table 2 Linkage of *Bcg* with *Lsh* in segregating backcross progeny

Population	<i>Bcg</i>		<i>Lsh</i>		
		log ₁₀ of BCG CFU	Type	Type	LDU
(B10.A × A)F ₁	1	4.43	r	r	8
	2	3.69	r	r	0
	3	4.11	r	r	29
	4	3.97	r	r	0
	5	3.67	r	r	14
B10.A	1	5.91	s	s	1,782
	2	5.70	s	s	1,297
	3	5.62	s	s	1,396
	4	5.76	s	s	1,664
	5	5.56	s	s	644
F ₁ × B10.A backcross	1	3.85	r	r	0
	2	4.06	r	r	16
	3	5.72	s	s	575
	4	4.26	r	r	6
	5	5.61	s	s	791
	6	3.88	r	r	26
	7	5.36	s	s	210
	8	5.90	s	—	Dead
	9	3.82	r	r	43
	10	4.03	r	r	10
	11	3.45	r	r	39
	12	5.32	s	s	640
	13	5.15	s	s	1,332
	14	4.14	r	r	8
	15	5.08	s	s	441
	16	5.92	s	s	955
	17	5.46	s	s	550
	18	4.32	r	r	15
	19	4.11	r	r	13
	20	5.68	s	s	548
	21	5.36	s	s	464
	22	6.13	s	s	580
	23	6.12	s	s	214
	24	4.08	r	r	6
	25	3.70	r	r	14
	26	6.45	s	s	587

r = Resistant; s = susceptible. *Bcg* typing performed on spleens 3 weeks after intravenous infection with 3×10^4 CFU BCG⁵. *Lsh* typing performed on livers 2 weeks after intravenous infection according to Bradley⁸ and expressed as Leishman-Donovan units (LDU) per liver according to Actor¹⁵. An animal typed as *Bcg*^r (resistant) if the $\log_{10}$ BCG count in the spleen was <4.5 (2 s.d. above the mean BCG count of resistant (B10.A × A)_{F1} controls, 95% confidence limit). An animal typed as *Lsh*^r (resistant) if the LDU in the liver was <46 (2 s.d. above the mean LDU count of resistant (B10.A × A)_{F1} controls, 95% confidence limit). The LDU counts were generally somewhat lower and their range was greater in *F1* × B10.A backcross susceptible mice than in B10.A parents. Although the *Lsh* gene undoubtedly dominates the regulation of resistance to *L. donovani* these results suggest that other genes also have some effect in controlling the acute growth rate of this parasite. Same conclusion was already reached by Bradley⁸.

nor previous infection with BCG influenced the *Lsh* typing. These manipulations did not change the susceptibility to *L. donovani* of B10.A control mice, nor did they change the resistance to *L. donovani* to (B10.A × A)_{F1} control mice. Of 26 [(B10.A × A)_{F1} × B10.A] backcross animals, 12 were typed as resistant to BCG and 14 were typed as susceptible to BCG, consistent ($\chi^2 = 0.15$, $P > 0.7$) with the hypothesis that the trait of resistance to BCG is under monogenic, dominant control⁵. Furthermore, all 12 of the BCG-resistant backcross mice proved to be resistant to *L. donovani*, and all of the BCG-susceptible backcross mice proved to be susceptible to *L. donovani*. A similar experiment to establish the linkage of *Ity* with *Bcg* could not be done since splenectomy influenced the *Ity* typing: splenectomized mice of *Ity*^r strains became

Table 1 *Bcg*, *Ity* and *Lsh* typing of inbred strains of mice

Strain	<i>Bcg</i> [*]	<i>Ity</i> [†]	<i>Lsh</i> [‡]
A/J	3.90 ± 0.40 r	r	r
AKR/J	3.95 ± 0.21 r	r	r
C3H/HeN	3.95 ± 0.15 r	r	r
C3H/HeJ	3.71 ± 0.11 r	r§	r
C57BR/J	4.12 ± 0.25 r	r	r
C57L/J	4.34 ± 0.22 r	r	r
CBA/J	4.21 ± 0.13 r	r	r
DBA/2J	3.93 ± 0.18 r	r	r
129/J	4.13 ± 0.70 r	—	r
BALB/cJ	5.80 ± 0.08 s	s	s
DBA/1J	6.18 ± 0.13 s	s	s
C57BL/6J	5.75 ± 0.09 s	s	s
C57BL/10J	6.05 ± 0.20 s	s	s
CE/J	5.67 ± 0.14 s	—	s

r, Resistant; s, susceptible. All mice except C3H/HeN were purchased from the Jackson Laboratory, Bar Harbor, Maine. C3H/HeN mice originating at The Charles River Breeding Laboratories, Wilmington, Massachusetts, were purchased from Canadian Breeders, St Constant, Quebec.

^{*} $\log_{10}$ CFU of BCG ± s.d. in spleens 3 weeks after infection with 10^4 CFU BCG.

[†] *Ity* typing taken from Robson and Vas¹³, Plant and Glynn⁷ and O'Brien *et al.*³.

[‡] *Lsh* typing taken from Bradley *et al.*⁸.

§ C3H/HeJ is *Ity*^r (ref. 14) although phenotypically susceptible to *S. typhimurium* due to the presence of *Lps*^d mutation in that strain.

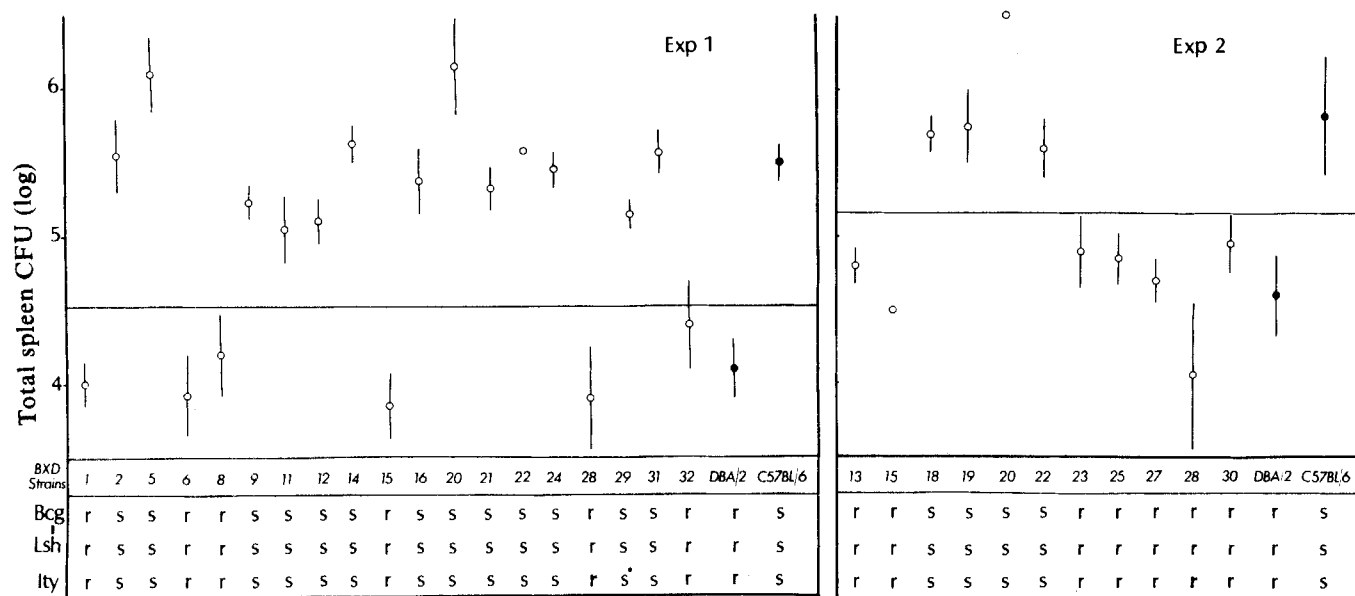


Fig. 1 Typing of BXD RI strains for resistance to BCG. Two to eight animals of each recombinant inbred strain and six animals of control DBA/2 and C57BL/6 strains were infected with $2-3 \times 10^4$ CFU BCG in two separate experiments. Geometric means $\pm$ s.d. of viable BCG in the spleens of infected animals were calculated 3 weeks after the infection. The horizontal line represents 95% confidence limit (two standard deviations above the mean of BCG numbers in the spleens of resistant DBA/2 progenitors) for typing of strains as *Bcg*^r (below the line) or *Bcg*^s (above the line). *Lsh* type of BXD strains was taken from Bradley *et al.*² except for BXD-28 and BXD-20. *Lsh*^s allele of these strains, identified by Plant *et al.*⁴, was confirmed by us according to the criteria described in Table 2 (mean LDU $4,445 \pm 57$ got BXD-18 and $7,059 \pm 1,066$ for BXD-20, with values of LDU 116 ± 25 for *Lsh*^r control and $11,355 \pm 2,455$ for *Lsh*^s control in that particular experiment). *Ity* types of BXD strains were taken from O'Brien *et al.*³ except for BXD-28 and BXD-29. Designation of *Ity* alleles of these two strains is taken from Plant *et al.*⁴. The *Ity*^r type of BXD-28 strains was confirmed according to the criteria described in Table 3, by typing (BXD-28 $\times$ B10.A)F₁ mice (7/7 animals survived the typing dose of 10^4 *Salmonella* i.v.). r, Resistant; s, susceptible.

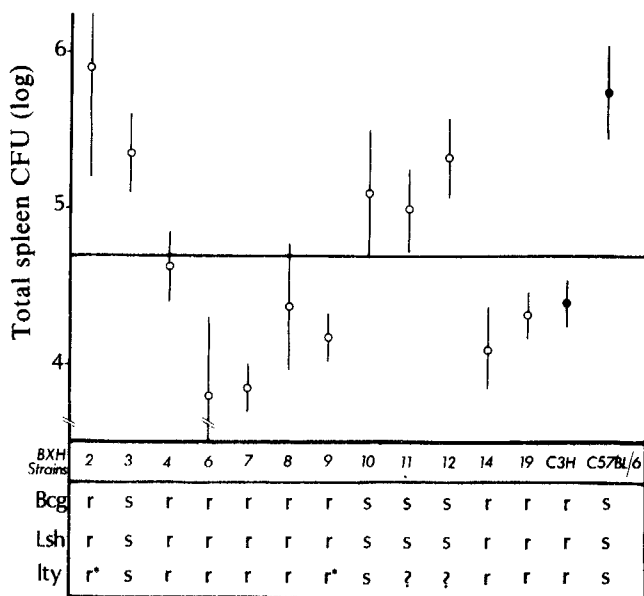


Fig. 2 Typing of BXH RI strains for resistance to BCG. Details as for Fig. 1, except for the resistant progenitor which in this case is C3H/HeJ. r, Resistant; s, susceptible; ?, some of the BXH strains were not *Ity* typed because of the interfering influence of the *Lps*^d mutation in the genome of C3H/HeJ progenitor; *, *Ity* type established by A. D. O'Brien (personal communication). Note that BXH-2 is designated as *Bcg*^r although phenotypically susceptible for BCG (see explanation in the text). BXH-10 is designated as *Lsh*^s according to the criteria described in Table 2 (mean LDU 990 ± 290), in variance with Bradley *et al.*². *Ity*^s allele of BXH-10 strain was established by typing (BXH-10 $\times$ B10.A)F₁ mice, according to the criteria described in Table 3 (0/8 animals survived the typing dose of 10^4 *Salmonella* i.v.).

phenotypically susceptible to *S. typhimurium* infections. The reverse experiment (*Ity* typing first, with subsequent *Bcg* typing) also proved to be technically impossible. *Ity*-*Bcg* linkage was, therefore, established by progeny testing which eliminated the necessity of splenectomy and of double infection of an individual animal. Twenty-five randomly chosen males of the [(B10.A $\times$ A)F₁ $\times$ B10.A] backcross generation were mated to *Bcg*^s-*Ity*^s females of B10.A strain. The resulting progeny were *Ity*-typed and the corresponding males of the parental generation were subsequently *Bcg*-typed. We reasoned that, if the *Bcg* and *Ity* genes were identical or closely linked, the offspring of BCG-susceptible (*Bcg*^s/*Bcg*^s, homozygous) backcross males mated with *Bcg*^s-*Ity*^s females should breed true and all should type as *Salmonella*-susceptible (*Ity*^s/*Ity*^s, homozygotes). The offspring of BCG-resistant (*Bcg*^r/*Bcg*^s, heterozygous) backcross males and B10.A females should segregate between *Salmonella*-susceptible (*Ity*^s/*Ity*^s, homozygotes) and *Salmonella*-resistant (*Ity*^r/*Ity*^s, heterozygotes) progeny.

Due to a laboratory accident, eight of the backcross males (BC 18-BC 25) were lost before they could be *Bcg*-typed. Fortunately, two litters of their mating with B10.A females were available and the *Bcg* type of the males could, therefore, be deduced from *Bcg* typing of the litter. When the results of *Bcg* and *Ity* typing were matched, a *Bcg*^r-*Ity*^r or *Bcg*^s-*Ity*^s concordance was found among 24 out of 25 first backcross males. The only discordance, BC 22, (*Bcg*^s(?) - *Ity*^r) is inconclusive because the *Bcg* typing was based on only six progeny, and could have resulted from chance segregation ($P = 0.016$), even if the BC 22 male had been *Bcg*^r/*Bcg*^s.

Collectively, the data presented here clearly establish that *Bcg*, the gene controlling resistance to *Mycobacterium bovis* BCG, maps on chromosome 1 and that it is either identical to, or linked with, the gene(s) controlling resistance to *S. typhimurium* and *L. donovani*. Since no conclusive recombination between the *Bcg*, *Ity* and *Lsh* alleles was found by back-

Table 3 Linkage of *Bcg* with *Ity* by progeny testing

	BCG infection*		Salmonella infection†	
	Total spleen BCG (log)	No. resistant/total B10.A × BC progeny§	<i>Ity</i> type	No. resistant/total B10.A × BC progeny§
BC 1‡	4.11		r	2/7
BC 2	5.72		s	0/14
BC 3	4.92		r	5/13
BC 4	5.62		s	0/3
BC 5	5.40		s	0/7
BC 6	3.94		r	3/7
BC 7	4.53		r	3/7
BC 8	3.94		r	5/10
BC 9	4.22		r	4/5
BC 10	5.33		s	0/8
BC 11	4.68		r	4/8
BC 12	5.54		s	0/8
BC 13	4.58		r	4/12
BC 14	4.54		r	9/12
BC 15	5.64		s	0/4
BC 16	4.36		r	6/10
BC 17	4.54		r	8/12
BC 18		0/7	s	0/8
BC 19		1/5	r	2/5
BC 20		1/4	r	5/8
BC 21		1/5	r	4/9
BC 22		0/6	s	4/11
BC 23		3/4	r	5/9
BC 24		0/6	s	0/9
BC 25		0/7	s	0/6
B10.A	5.60–5.92		s	0/25
(B10.A × A) _{F1}	3.93–4.64		r	25/25

* Animals typed 3 weeks after intravenous infection with 2.5×10^4 CFU BCG. Resistant (r): $\log_{10}$ BCG count in the spleen $< \text{mean} \pm 2 \text{ s.d.}$ of resistant control, 95% confidence limit. Any animal with $\log_{10}$ BCG count above that value typed as susceptible (s).

† Animals typed 1 week after intravenous infection of 10^4 *Salmonella typhimurium* as r = resistant (survivors) or s = susceptible (dead).

‡ BC = [(B10.A × A)_{F1} × B10.A].

§ B10.A × BC = B10.A × [(B10.A × A)_{F1} × B10.A]. Two successive litters of the same parental pair were typed in some instances.

|| Range of control values in 5 individual experiments which constitute the table.

cross analysis, and because of the full concordance of SDP of resistant and susceptible alleles of *Bcg*, *Ity* and *Lsh* among the total of 38 RI strains examined, these data suggest that the genetic control of resistance to *M. bovis* BCG, *S. typhimurium* and *L. donovani* is exerted by a single gene with pleiotropic effect. An alternative interpretation, that of a complex of closely-linked genes³, seems less likely although it obviously cannot be ruled out completely. The fact that the susceptible allele is recessive, and that it is limited to a relatively few inbred strains, is more compatible with the possibility that the susceptibility gene might represent a mutation and be of a recent origin, rather than being a variant of a polymorphic gene that occurs in natural populations. That being the case, a single mutation from resistance to susceptibility would be more likely than the accumulation of three independent mutations to susceptibility genes (*Bcg*^s–*Lsh*^s–*Ity*^s) in coupling on the same chromosome.

The phenotypic expression of the gene in question is, so far, recognized *in vivo* only: in each case the resistant allele expresses itself as slow (or null) net growth rate of the particular microorganism in the liver or spleen^{5,10,11} early in the course of infection, most probably before the specific immune response is established. It seems likely that the cellular expression of the resistant allele(s) is a bacteriostatic effect of the intracellular milieu of the parasitized macrophage¹², although no clear *in vitro* data are available as yet. The normal (resistant) allele might have other functions (either in macrophages or other cell types) but it would not be surprising if its chief function were the defence against intracellular parasites. Accordingly, the discovery of the mechanism by which this gene exerts its effect could reasonably be expected to shed considerable light on how macrophages help to control infections by organisms of medical significance. A consideration of common features (for example,

biochemical or nutritional requirements for multiplication) of these three taxonomically distinct pathogens (*L. donovani*, *S. typhimurium* and *M. bovis*) could provide a starting point in this endeavour. We propose that natural resistance to other infections, such as tuberculosis and leprosy, to name but two, is also regulated by this gene. The biological implications of these findings for identification of genetically susceptible individuals and for the development of appropriate strategies for their protection and treatment are obvious.

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Are the *Lsh* and *Ity* disease resistance genes at one locus on mouse chromosome 1?

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Ity, a gene controlling natural resistance to *Salmonella typhimurium* infection^{1,2}, and *Lsh*, which controls innate resistance to *Leishmania donovani* infection³, are both located on chromosome 1 of the mouse^{4,5} and it has been suggested⁶ that they might be identical. O'Brien *et al.*⁷ examined salmonella resistance in recombinant inbred (RI) strains of mice, and observed discordant responses in three strains (BXD-18, BXD-20, BXD-29) from one progenitor strain combination (C57BL/6J × DBA/2J) when compared with the published *Lsh* typing⁵. On the basis of this and additional information on salmonella resistance in (C57BL/6J × BXD-18)F₁ and [(C57BL/6J × DBA/2J) × BXD-29] backcross mice, it was concluded that *Ity* and *Lsh* are closely linked but distinct genetic loci. We have now re-examined both leishmania and salmonella resistance in larger numbers of these putative recombinant strains and in various hybrid and backcross generations, and our results indicate that BXD-18 and BXD-20 retype as *Lsh*^s and BXD-29 as *Ity*^s. Hence there is no longer any discordance between *Lsh* and *Ity* typings for the RI strains. The results do, however, suggest additional modifying genetic control of salmonella resistance in BXD strains involving at least two other genetic loci. There is therefore no clear evidence to demonstrate that *Ity* and *Lsh* are not the same genetic locus.

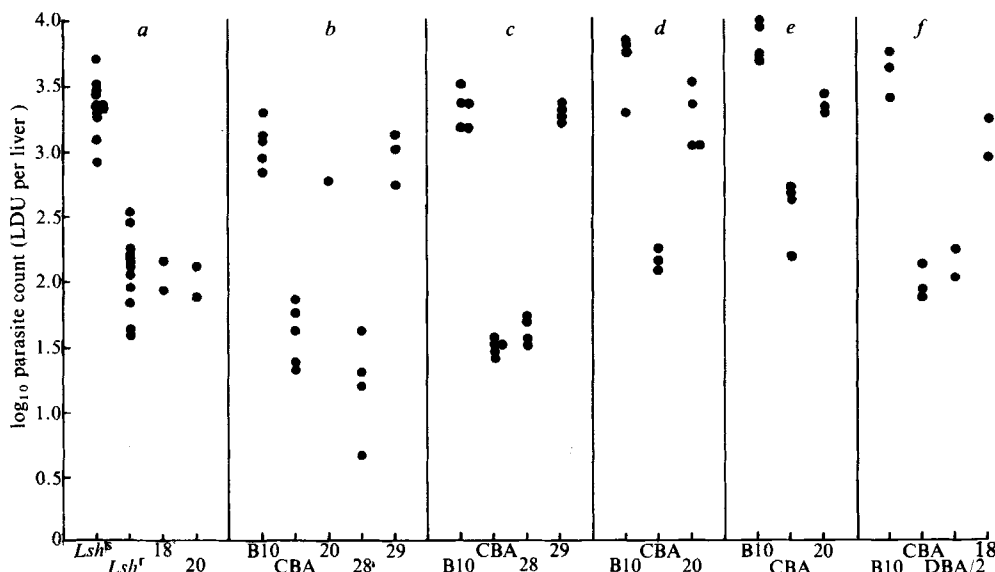
Initially we examined leishmania and salmonella resistance in the three BXD strains thought to differ at *Lsh* and *Ity* and in an additional strain (BXD-28) found earlier⁷ to be intermediate in its susceptibility to *S. typhimurium* TML strain. (The BXD mice used in our experiments were derived from stock

sent by Dr Benjamin Taylor of the Jackson Laboratory.) Salmonella resistance was further examined in the progeny of BXD-29 mice mated with stock DBA/2, C57BL and (C57BL/10ScSn × DBA/2)F₁ mice kept at the Wright Fleming Institute at St Mary's Hospital Medical School, London. C57BL/10ScSn and CBA/Ca mice used as susceptible and resistant controls respectively for *Lsh* typing were obtained from Olac 1976 Ltd. Mice were typed individually for susceptibility to either *S. typhimurium* C5 or *L. donovani* infection as previously described^{2,3}. In addition, (C57BL/6J × BXD-29)F₁ mice bred at the Jackson Laboratory were tested for resistance to *S. typhimurium* C5 (ref. 2) and TML (ref. 7) in the two laboratories.

Two BXD-18 and eight BXD-20 mice all typed as *Lsh*^s (Fig. 1). In each case, the original typing of *Lsh*^s by Bradley *et al.*⁵ was based on examination of two mice whose parasite counts fell well within the *Lsh*^s range (Fig. 1, actual counts previously unpublished). This suggests that BXD-18 and BXD-20 had not proceeded to fixation for either allele at *Lsh* at that time. Our more recent *Lsh*^s typings are now consistent with the earlier⁷ unequivocal *Ity*^s typings for BXD-18 and BXD-20 and thus eliminate two of the putative recombinants.

Lsh typing of eight BXD-28 and seven BXD-29 mice (Fig. 1) confirm the original typings (*Lsh*^s and *Lsh*^s respectively) of Bradley *et al.*⁵. In the case of BXD-28, the geometric mean spleen count of 4.61 ± 0.98 obtained after re-examining 20 mice with *S. typhimurium* C5 agrees closely with the mean of 4.46 obtained in the earlier⁷ work with the TML strain of *S. typhimurium*. For BXD-29 mice, however, the geometric mean spleen count of 5.50 ± 0.95 based on examination of 34 mice with the C5 strain is significantly higher than the mean of 3.9 obtained after examination of only 5 mice with the TML strain, which led to the earlier⁷ typing of BXD-29 as *Ity*^s. On the basis of our original criteria⁴ for typing individual mice using 10³ *S. typhimurium* C5 and day 10 organ counts, 22 of 34 BXD-29 mice with spleen and liver counts >10⁵ type as *Ity*^s. The remainder were high in the *Ity*^s range with the geometric mean 2 logs higher than that for DBA/2 *Ity*^s controls. Our combined C5 and TML results (Fig. 2), which show 16 of 17 (C57BL/6J × BXD-29)F₁ and 26 of 28 (BXD-29 × C57BL)F₁ mice with spleen and liver counts clearly in the susceptible (>10⁵) range, support the hypothesis that BXD-29 is, in fact, *Ity*^s. This would explain the previous finding⁷ that 58% of BXD-29 mice tested died by day 30 when given only 25 organisms of the TML strain.

Fig. 1 *Lsh* typing experiments for RI (BXD-18, 20, 28, 29) and control *Lsh*^s (C57BL/10ScSn or B10) and *Lsh*^r (CBA/Ca and DBA/2) strains of mice. Mice were injected with 1–5 × 10⁷ amastigotes of the L82 strain of *L. donovani* and examined 15 days later. The number of parasites per 500 liver cell nuclei was counted from Giemsa-stained impression smears and the results expressed as 'Leishman-Donovan units' (LDU) as described previously¹². Counts for individual mice are shown for six separate typing experiments. *a* Shows actual counts for mice used in the original *Lsh* typing experiments of Bradley *et al.*⁵. Counts for BXD-18 and BXD-20 mice are compared with the range of *Lsh*^s and *Lsh*^r counts obtained for all BXD strains examined in that experiment. Both clearly fell well within the *Lsh*^r range of counts. *b–f* Show the results of our more recent typing experiments. Counts for BXD-28 and BXD-29 mice (*b, c*) fell clearly within control *Lsh*^r and *Lsh*^s ranges respectively. Counts for BXD-18 and BXD-20 mice (*b, d–f*) always fell towards the lower end of the *Lsh*^s range of counts, irrespective of parasite dose. Although this difference was not always significant in individual experiments, it does suggest some additional modifying genetic control of *Lsh*^s counts. B10, BXD-18 and BXD-20 counts all differed significantly from control *Lsh*^s counts ($P < 0.001$) and, in each experiment, there was at least a 0.5 log₁₀ LDU separation between the lowest susceptible and highest resistant counts. The retyping of BXD-18 and BXD-20 as *Lsh*^s has recently been confirmed in another laboratory¹⁰ where parasite counts again fell at the lower end of the susceptible range of counts.



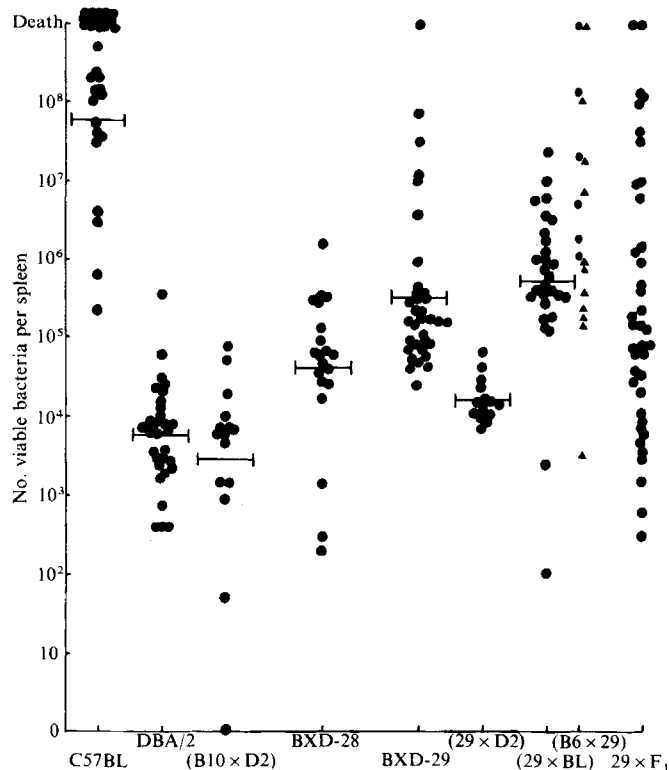


Fig. 2 *Ity* typing experiments for RI (BXD-28, 29) strains and the F_1 hybrid combinations: (C57BL/10ScSn $\times$ DBA/2) or (B10 $\times$ D2); (BXD-29 $\times$ DBA/2) or (29 $\times$ D2); (BXD-29 $\times$ C57BL) or (29 $\times$ BL); (C57BL/6J $\times$ BXD-29) or (B6 $\times$ 29); and [BXD-29 $\times$ (C57BL/10ScSn $\times$ DBA/2) F_1] or (29 $\times$ F_1). Mice were injected subcutaneously with 10^3 *S. typhimurium* strain C5 (●) or TML (▲) and examined 10 or 9 days later respectively. Viable bacteria in spleens and livers were estimated as previously described^{2,4,7}. Bacterial loads in the livers agreed closely with the viable bacterial counts per spleen shown for individual mice. Dead mice were estimated at 10^8 bacteria per spleen in calculating the $\log_{10}$ geometric mean spleen counts indicated for each group. The RI strains and hybrids are compared with control *Ity*⁺ (C57BL) and *Ity*⁻ (DBA/2) strains.

A range of sensitivity from highly susceptible (10^7 – 10^8 counts or death by day 10) through intermediate (10^5 – 10^6) to highly resistant (10^2 – 10^4) was observed in [BXD-29 $\times$ (C57BL/10ScSn $\times$ DBA/2) F_1] backcross mice (Fig. 2). This implies multigenic control additional to *Ity*. The possibility of additional genetic influence on the sensitivity to *S. typhimurium* infection in the intermediate BXD strains has already been considered⁷ and is consistent with the previous observations^{1,8,9} of increased susceptibility in the *Ity*⁻ DBA/2 progenitor strain compared with other *Ity*⁻ strains. A single additional gene bearing susceptible alleles in the DBA/2 progenitor and resistant alleles in the C57BL/6 progenitor is insufficient, however, to explain the present results. We propose, therefore, that at least two other genes are involved in modifying the phenotypic expression of *Ity* in the BXD intermediate strains.

We therefore conclude that it is not proved that *Ity* and *Lsh* are distinct genetic loci. The fact that no recombinants were detected among 22 RI strains from 4 other progenitor strain combinations⁷ supports our hypothesis that the progenitors of BXD are introducing additional genetic influence on the outcome of salmonella infections. The recent finding¹⁰ that *Bcg*, a gene controlling innate resistance to *Mycobacterium bovis* infection in mice,¹¹ types in concordance with the present *Lsh* and *Ity* typings for the BXD strains also supports our conclusions. We therefore favour the hypothesis that a single gene on chromosome 1 controls innate resistance and susceptibility to these three, and possibly other, intracellular infections via one mechanism, rather than a complex of tightly linked genes.

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Recognition of conformational determinants on H-2 by cytolytic T lymphocytes

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The specificity of cytolytic T lymphocytes (CTL) differs from that of B lymphocytes insofar as they exhibit a marked preference for recognition of major histocompatibility complex (MHC) encoded cell surface antigens. In murine systems, this is reflected both in the high frequency of CTL which recognize H-2 alloantigens^{1–5} and also in the requirement for recognition of syngeneic H-2 antigens in conjunction with cellularly presented foreign antigens, so called MHC restricted recognition^{6–10}. Various models for T-cell recognition have been proposed in an attempt to explain both this predisposition for MHC recognition and the basis for restricted recognition (reviewed in refs 10, 11). Most models have fallen into one of two general categories; those which attribute recognition to two independent combining sites on the T cell receptor(s), one specific for foreign antigens and one specific for MHC antigens; and those which accommodate recognition of both molecules using a single combining site which recognizes a neoantigenic determinant composed of a complex of both molecules. In order to understand the basis for determinant recognition, the specificity of individual clones of C57BL/6 CTL induced in response to a point mutation in the H-2K^b molecule has been analysed. Using a series of H-2K^b mutants as targets it was found that a single clone can recognize two or more mutants which, although different from each other with respect to the position of their amino acid substitutions, appear to have independently gained the same new determinant. This strongly suggests that CTL can respond to conformational nuances in self H-2 molecules as shared by these different K^b mutants. On this basis, it is proposed that MHC restricted recognition may represent the recognition of conformational alterations of self resulting from the interaction of MHC molecules and foreign antigens.

In order to approach questions concerning T-cell recognition, this laboratory has carried out a detailed analysis of the recognition of H-2 antigens by individual CTL clones^{12–15}. Most revealing in terms of probing the basis for determinant recognition have been the results of fine specificity analysis of monoclonal CTL stimulated as a result of a single amino acid substitution in the H-2K^b molecule, the B6.C-H-2^{bm11} anti-C57BL/6 response (bm11 anti-B6)¹³. A large proportion (25%) of such clones did not recognize any of the five different H-2K^b mutants which were used as targets. Considering that each of these mutants differed from the stimulating K^b molecule by only one

or two amino acid substitutions, and that in most cases each mutation occurred at different positions on the molecule¹⁶, it is unlikely that all of these different mutations could have resulted in an amino acid substitution in the particular determinant recognized by a single clone. A more likely explanation for such lack of recognition would be that, in some if not all mutants, these particular wild-type determinants have been lost due to conformational changes resulting from the mutation^{17,18}. In the case of antibody recognition of globular proteins, such a mechanism has previously been used to explain how the antigenic integrity of a particular determinant can be disrupted by a distal amino acid substitution¹⁹.

This suggests the possibility that a conformational alteration might also create new determinants which were, by analogy, in positions on the molecule different from the site of the amino acid substitution responsible for their creation. In support of this possibility, Melief and co-workers previously demonstrated that, although B6-anti-mutant CTL populations would most strongly react against the K^b mutant used as stimulator these CTL demonstrated detectable cross-reactivity on a variety of disparate K^b mutants¹⁸. This suggested different K^b mutants may have gained some similar determinants. A possible mechanism would be that each mutation creates its own set of conformational differences in the K^b molecule and that amino acid substitutions in different parts of the molecule could independently effect a conformational change which, fortuitously, resulted in a similar determinant.

The experiments described in this report constitute a test of this hypothesis. CTL clones of wild-type origin were stimulated in primary limiting dilution cultures against the bm11 mutant. It was reasoned that the wild-type responder would be tolerant of all K^b determinants retained by the bm11 mutant and, therefore, such clones should specifically recognize new determinants attributable to the bm11 mutation. Each clone was tested for cross-reactive recognition on a panel of targets which included the wild type B6, six other K^b mutants, and the allogeneic D2.GD target (K^d, D^b). Where known, the positions of the amino acid substitutions in each of these mutants is as follows: bm1, 155, and 156; bm3, 77 and 89; bm8, 23; bm9, 116 and 121; bm10, 165; and bm11, 77 (ref. 16).

Results obtained from panel analysis of 58 B6-anti-bm11 clones independently derived from 11 individual B6 responders

are presented in Table 1. Positive recognition refers to cross-reactive lysis which represents at least 60% of the value obtained on the bm11 target as indicated in the legend to Table 1. As expected, none of the B6 clones demonstrated specific recognition of the syngeneic B6 targets. Also, as expected on the basis of a shared mutation at amino acid position 77, the vast majority of bm11 specific clones (83%) also recognized the bm3 target. It is likely that the antigenic determinants recognized by the 17% which did not recognize bm3 are obscured or disrupted on the bm3 molecule as a result of the second mutation at position 89. Most interesting, however, is the observation that the majority of clones (62%) recognized not only targets which bear the mutation in position 77, but also recognize at least one of the other K^b mutants such as bm8, bm1 and bm9 which are identical to the wild type K^b in position 77, having their substitutions elsewhere. Moreover, of these 26 clones, 24% recognize at least two such H-2K^b mutants.

In Table 2, these data are presented as the fraction of clones that recognize each of these targets. The fraction of clones that cross-react on the third-party alloantigen, K^d on D2.GD, is comparable to the level of third-party reactivity usually seen in conventional allogeneic responses³⁻⁵ (5-10%). In contrast, some of the mutants, bm8, bm1, bm9, are recognized by a significantly greater proportion of clones than is usual for third party recognition. Based on these results, it may be concluded that different and often distal amino acid substitutions in an H-2 molecule can fortuitously result in antigenic determinants which if not identical, are at least similar enough to permit comparable T-cell recognition as reflected in the comparable degree of lysis of different mutant targets.

In view of the disparate positions of most of these mutations, it would appear unlikely that two different mutants could demonstrate significant cross-reactivity on the basis of a common 'foreign' determinant. The only candidates for a non-self antigen as perceived by the strain of origin would be determinants that encompass the substituted residues and it is unlikely these are cross-reactive since they would correspond to a different position on the molecule for each mutant. Therefore, these results strongly support the hypothesis proposed above, that conformational alterations of self H-2 antigens can result in immunogenic determinants which do not include the

Table 1 Panel analysis of B6 anti-bm11 CTL clones

Reactivity patterns	C57BL/6Kh	bm1	bm3	bm4	bm8	bm9	bm10	bm11	D2.Gd	No. of clones	% Of total
a	.	.	.	.	.	.	.	+	.	6	10.3
b	.	+	.	.	.	.	.	+	.	1	1.7
c	.	.	+	.	.	.	.	+	.	13	22.4
d	.	.	.	.	+	.	.	+	.	2	3.4
e	.	.	.	.	.	+	.	+	.	1	1.7
f	.	+	+	.	.	.	.	+	.	9	15.5
g	.	+	+	+	.	.	.	+	.	2	3.4
h	.	.	+	+	.	.	.	+	.	3	5.2
i	.	.	+	.	+	.	.	+	.	6	10.3
j	.	.	+	.	+	+	.	+	.	1	1.7
k	.	.	+	.	.	+	+	+	.	2	3.4
l	.	+	+	.	.	+	+	+	.	1	1.7
m	.	.	+	.	.	+	.	+	.	7	12.1
n	.	.	+	.	.	.	.	+	+	2	3.4
o	.	+	+	.	.	.	.	+	+	1	1.7
p	.	.	+	.	+	.	.	+	+	1	1.7

B6 splenic CTL precursors were stimulated *in vitro* under limiting dilution culture conditions in V bottom microtitre wells containing 5×10^5 irradiated B6 'helper' spleen cells, 5×10^6 irradiated bm11 stimulator cells, and varying numbers (1,000-6,000) of B6 responder cells in 0.2 ml of culture media as previously described¹². After 6 days in culture, a 60 μ l portion of each well was tested for cytolytic activity against conA stimulated bm11 targets. Such target cells were prepared and labelled with ¹²⁵I-iododeoxyuridine¹² and 1×10^4 cells were added to each well in 140 μ l of culture media containing 10% fetal bovine serum and 3% rat-T-cell growth factor which was a necessary addition to maintain maximum viability of these target cells during long assays (18-20 h). CTL clones obtained using responder cell numbers resulting in 20% or fewer positive wells were expanded¹² and tested for lytic activity against ⁵¹Cr-labelled bm11 target cells. Successfully expanded clones were assayed in duplicate on each of the indicated target cells. A clone was considered positive or negative for recognition of a particular target if its value for lysis was either >60% or <25% respectively of its value for lysis on bm11 targets. Clones which did not meet these criteria on all targets were considered ambiguous and not recorded above.

Table 2 Cross-reactive recognition of H-2K^b mutants by B6 anti-bm11 CTL clones

Strain	% Clones
bm11	100
bm3	83
bm1	24
bm9	21
bm8	17
bm4	9
bm10	5
D2.GD	7

amino acid substitution responsible for the alteration.

Based on these results, it is further proposed that the interaction of foreign antigens with H-2 encoded molecules can similarly result in conformational changes which may be immunogenic. A similar hypothesis has recently been proposed by Ohno²⁰. In order to conform with our views of restricted recognition, such interaction would need to fulfil the following requirements: (1) the same antigen should always result in the same conformation change in H-2, and (2) this interaction should be sufficiently stable to permit binding and triggering of T receptors that recognize the resultant novel H-2 conformation. It would then be possible to explain the appearance of antigen specificity and MHC restriction even though the receptor actually recognized 'altered self' and not antigen.

There are several observations which are consistent with such a model. First, there is much evidence for stability of H-2 antigen interactions as measured either by co-capping or co-purification of these two molecules²¹⁻²⁵. Second, it has proven much easier to block antigen specific CTL or MLC using anti-H-2 antibody reagents as opposed to using antibody specific for the foreign antigen *per se*²⁶⁻²⁹. This is most consistent with an indirect role for antigen in receptor recognition. Third, it has been reported by several laboratories that influenza specific CTL are more cross-reactive than influenza specific antibody³⁰⁻³². This is again most readily accommodated by a model which predicts that only some foreign determinants potentially contribute to the specificity of a CTL response, in particular, those which effect the interaction between H-2 and the foreign molecule. Finally, this model presents a structural basis for the well documented cross-reactivity against allo-antigen of antigen specific T cells, since both types of recognition would involve altered-self MHC determinants³³⁻³⁶.

The implication of this hypothesis which may be most difficult to accept is the burden placed upon the H-2 molecule by the fact that the diversity of all possible H-2 determinants would have to be sufficient to account for antigen specificity. However, if the low frequency of CTL precursors specific for non-MHC antigens, such as virus and minor antigens^{5,37}, reflects a correspondingly small number of different determinants created by an antigen H-2 interaction, then the chances that a second antigen would create the same determinants may be relatively slim. In contrast, in essentially all responses where the H-2 antigen is itself structurally altered, many new determinants are created, thereby greatly increasing the likelihood of cross reactivity. This would account for the observation presented in this report.

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Cytogenetic mapping of the duplicated segment of chromosome 12 in lymphoproliferative disorders

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Recently, an extra chromosome 12 has been reported to occur with high frequency in peripheral blood lymphocytes from patients with chronic B-cell lymphocytic leukaemia (B-CLL)^{1,2}. Here we report a unique chromosomal abnormality, which suggests that the segment q13 to q22 on chromosome 12 carries the important genes that are duplicated in those lymphoproliferative disorders characterized by trisomy for this chromosome.

Cytogenetic studies were performed on peripheral blood lymphocytes, bone marrow cells and spleen cells from a 56 year-old man with a lymphocytic non-Hodgkin lymphoma. The lymphoma was found in the spleen, bone marrow, and peripheral blood. The white blood cell count was 9.3×10^9 per litre and a differential count showed that 74% of the cells were lymphocytes. Surface membrane immunofluorescence studies revealed a monoclonal B-cell proliferation with a phenotype $\mu\kappa$ when tested with fluorescein-conjugated F(ab')₂ fragments of goat anti-human μ , γ , δ , κ , and λ antisera. Microscopy of the spleen and small lymph nodes showed a mixed lymphocytic-histiocytic lymphoma (Rappaport classification³) or polymorphic immunocytoma (Kiel classification⁴).

Table 1 Metaphase analysis of lymphocytes from a patient with chronic B-cell lymphocytic leukaemia

Material	Mitogen	No. of metaphases analysed	No. of abnormal metaphases	Karyotype
Peripheral blood	EBV	17	2	46XY;
	LPS	11	3	46XY, t(10;10)
	PHA	21	1	(pter; q24),
Bone marrow	EBV	6	2	dup(12)
Spleen	EBV	5	4	(q13-q22)
	LPS	3	1	
Total		63	13	

Mitogenic stimulation of isolated peripheral blood lymphocytes was performed with Epstein-Barr virus (EBV), lipopolysaccharide (LPS) from *Escherichia coli*, and phytohaemagglutinin (PHA)¹. Cultures for cytogenetic analysis were collected on day 4. Metaphase preparations were prepared by conventional methods and the preparations stained according to the Q-banding technique⁵. The results of the cytogenetic analyses are shown in Fig. 1 and Table 1. Of 63 metaphases, 13 had the same chromosomal abnormality. One chromosome 12 had an extra part attached at band 22, which was identical with the part q13→qter of a normal chromosome 12. The other chromosome 12 was normal. Thus, the region q13→q22 was duplicated. In addition to this abnormality, a balanced reciprocal translocation between the two chromosomes 10 was found in all metaphases carrying the abnormal chromosome 12.

As shown in Fig. 2, the observed abnormality of chromosome 12 has probably arisen through chromatid exchange. It is probable that a breakpoint occurred on one chromatid of one chromosome 12 at q13 and another breakpoint on the other chromosome 12 at q22. The two chromatids were then exchanged. Alternatively, there may have been three breakpoints, two on one chromatid of one chromosome 12 at q13 and q22, and one on q13 or q22 on one chromatid of the other chromosome 12. The chromatid part, q13→q22, on the first chromosome was then inserted at the breakpoint of the second chromosome 12. Of the possible abnormal karyotypes that can appear, irrespective of the mechanism, only alternative *b* in Fig. 2 was found. Normal karyotypes were also found, but these were probably due to the presence of normal nonmalignant lymphocytes in the peripheral blood¹.

The results show that the essential genes that tend to be duplicated during leukaemogenesis leading to lymphoproliferative B-cell disorders characterized by trisomy of chromosome 12, are probably located on the segment q13→q22.

Other experimental work on murine lymphomas has also indicated that only part of a trisomic chromosome is crucial for the neoplastic transformation⁶. Mouse T-cell lymphomas are characterized by trisomy 15, but only genes located distal to band T6 are important for leukaemogenesis. Furthermore, in certain human myeloproliferative disorders characterized by trisomy of chromosome 1, there are indications that only the segments q23→q25^{7,8} or q25→q32⁹ are associated with disease. Thus, in lymphoproliferative disorders (and probably also other haematopoietic diseases) characterized by trisomic chromosomes, the important genes to be duplicated for disease development are probably located on a very small, specific part of the chromosome. In human lymphoproliferative disorders characterized by trisomy 12, these genes seem to be located on the segment q13→q22. So far mapping of chromosome 12 has only located the loci for a few enzymes and antigens to the long arm of this chromosome, that is mitochondrial synthetase,



Fig. 1 Karyotype of a peripheral blood lymphocyte stimulated by EBV, showing duplication of the segment q13→q22 on chromosome 12. There is also a reciprocal balanced translocation between the two chromosomes 10.

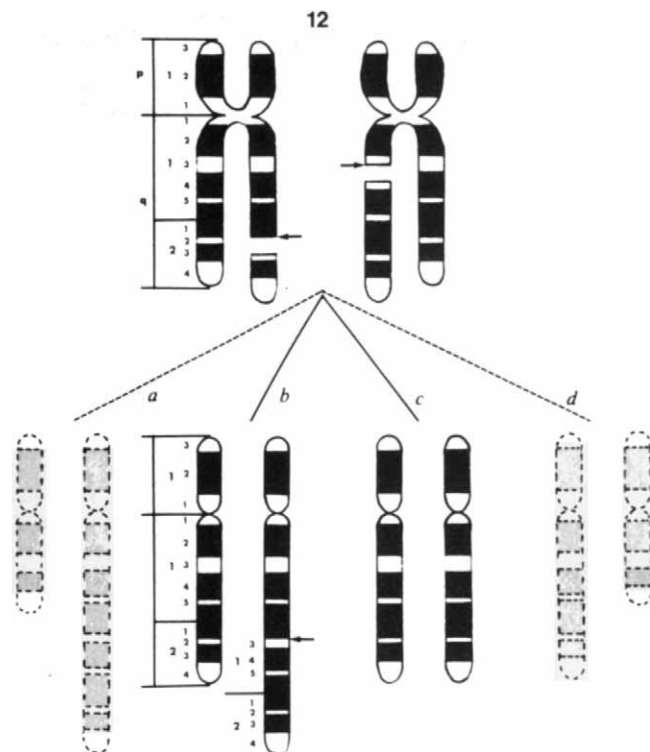


Fig. 2 Possible mechanism of development of the chromosomal abnormality. Only alternatives *b* and *c* were found.

serine hydroxymethyltransferase, branched-chain amino acid transferase-1, and surface antigen 8 (ref. 10). Further studies of these enzymes in lymphoproliferative B-cell disorders may elucidate whether any part of the q13→q22 region on chromosome 12 is also involved in patients where no gross cytogenetic abnormalities are found.

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Two distinct forms of surface antigen gene rearrangement in *Trypanosoma brucei*

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African trypanosomes express different surface antigens sequentially in their mammalian hosts¹. Expression of different variable surface glycoprotein (VSG) antigens is associated with rearrangements of genomic DNA^{2–5}. Several laboratories have reported that expression of some VSG genes is accompanied by the appearance of a duplicated copy of the genes at a new location in the genome^{3,4}. However, in a series of sequentially related *Trypanosoma brucei* clones from a different stock we were unable to detect duplication of one VSG gene (ILTAT 1.2, B) in expressing clones. Two copies of this gene were found

in expressing and non-expressing clones. Apparent insertions and deletions occurred near the 3' ends of both gene copies, but could not be correlated with expression^{2,5,6}. We report here the observation of both types of rearrangement for different VSG genes in the same series of trypanosome clones. Thus the different types of rearrangement are not a function of different trypanosome stocks, but of different VSG genes within a stock.

The trypanosome clones used in this study were derived from a single clone (clone A, expressing VSG A) from *T. brucei* stock 227 (ref. 7). Clone B was derived from a relapse infection of clone A and clones C and D were cloned from the first-relapse populations in normal mice infected with clone B. Trypanosomes grown in lethally irradiated rats were checked by immunofluorescence for homogeneity of VSG expression⁷ before isolation of RNA or DNA.

The construction and identification of plasmids pcBB1 and pcBC1 containing mRNA sequences encoding VSGs B (ILTAT 1.2) and C (ILTAT 1.3), respectively, have been described elsewhere⁸. Plasmid pcBA1 was constructed and initially identified in a similar manner. That pcBA1 contains VSG A coding sequences is substantiated by two further criteria: it contains 3'-coding and non-coding sequence homologies with other VSG mRNAs⁹, and hybridizes to RNA found only in trypanosome clone A. This is shown in Fig. 1, where pcBA1 was hybridized to total RNA from trypanosome clones expressing VSGs A, B, C and D (ILTAT 1.4). The inserted sequences of pcBA1 are present only in the trypanosome clone expressing VSG A. The only known clone-specific mRNA is that encoding the VSG expressed¹⁰. In addition to the major band at 2 kilobases (kb), the expected size of mature VSG mRNA, pcBA1 also hybridizes to three faint bands of 3.2, 3.6 and 4 kb, in clone A RNA only. As electrophoresis was done in stronger denaturing conditions¹¹, these bands may represent incompletely processed precursors of the VSG A mRNA. Such precursor RNAs have not been described previously for other VSGs.

The experiment shown in Fig. 2 characterizes the rearrangements affecting the genes for VSGs A, B, and C. Complementary DNA sequences encoding each of the VSGs

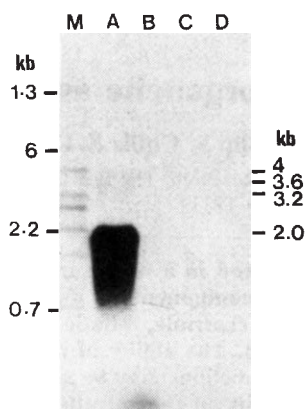


Fig. 1 Clone specificity of the cDNA sequence in pcBA1. Total RNA from four trypanosome populations homogeneously expressing different VSGs (A, B, C and D) was prepared by dissolving 10^{10} trypanosomes in 10 ml of 60% guanidinium thiocyanate, 50 mM sodium citrate pH 7.0, 0.5% sarcosyl. This was layered over 1.5 ml cushions of 5.7 M caesium chloride containing 50 mM EDTA pH 7.5, and centrifuged at 30,000 r.p.m. for 6 h in a Bechman SW 50.1 rotor. DNA remained at the interface and RNA was recovered from the pellet after careful removal of the supernatant and cushion; ~ 2.5 μ g from each clone was denatured with glyoxal, electrophoresed, transferred to nitrocellulose, and hybridized with radioactive pcBA1 as described by Thomas¹¹. The filter was washed twice in $0.1 \times$ SSC, 0.1% SDS at 50 °C for 15 min before autoradiography. The mRNA band was deliberately over-exposed to show the complete specificity of hybridization at this stringency, and to reveal the three faint high molecular weight bands in clone A. Molecular weight markers are shown under column M.

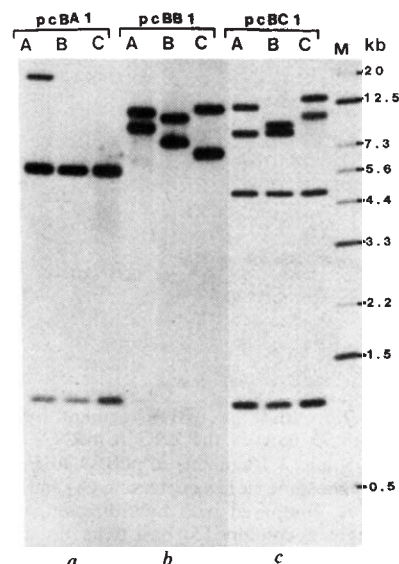


Fig. 2 Comparison of rearrangements affecting the genes for VSGs A (ILTAT 1.1), B (ILTAT 1.2) and C (ILTAT 1.3). DNA from trypanosome clones expressing each of these VSGs was digested with *HincII* (for hybridization to pcBA1 and to pcBC1) and *SalI* (for hybridization to pcBB1). These enzymes do not have cleavage sites in the cDNAs to which their digests were hybridized. Fragments were separated by electrophoresis on a single gel and transferred to nitrocellulose in $20 \times$ SSC as described elsewhere¹⁴. Cut sections of the filter were hybridized, in conditions described elsewhere¹⁴, to plasmids pcBA1 (a), pcBB1 (b) and 1,100-bp 5' fragment of pcBC1 (c), which had been labelled with 32 P by the procedure of Rigby *et al.*¹⁵. The markers (M) are fragments of known size which hybridize to pBR322 sequences in the pcBC1 probe. The filter sections were washed twice in $0.1 \times$ SSC, 0.1% SDS at 65 °C for 1 h, dried, and then realigned for exposure to X-ray film.

were hybridized to DNA from trypanosome clones expressing the three antigens. The enzyme used in each case did not cut the cDNA sequence of the VSG probe used. Thus, if there are no introns in the gene, each hybridizing band visualized implies a distinct region that is homologous with the probe.

For pcBA1, hybridizing fragments were identical in size in each clone except for the presence of an extra, larger fragment in DNA from clone A. A different result was seen for the other two probes. There was no additional fragment in the DNA from the cell clones expressing VSG B or C, as there was neither an extra band nor an increase in relative intensity of any band. Although the number of fragments hybridizing to each cDNA was the same for all trypanosome clones, there were large differences in the sizes of two of the hybridizing fragments, even when DNA from two non-expressing clones was compared.

Gene rearrangements were observed for all three cDNAs studied. Where an extra gene fragment was detected in the clone expressing the VSG encoded by the probe sequence (A), the remaining copies of the gene did not undergo rearrangements in different clones (Fig. 2). These characteristics of the VSG A gene rearrangement parallel observations by others using different VSG genes which are duplicated and transposed when expressed^{3,4}.

The experiment shown in Fig. 3a, using another enzyme that does not cut the cDNA, also demonstrates an extra fragment hybridizing to pcBA1 in clone A. This fragment hybridizes strongly to probe fragments from either end of the cDNA, indicating that it contains both ends of the cDNA sequence.

For other VSG genes which are duplicated and transposed when expressed, the duplicated segment extends from a point within the 3' end of the mRNA sequence to a point beyond its 5' end¹². When enzymes cutting inside a transposed segment were used to observe duplicated genes, single bands were visualized with increased intensity. For VSG A, this was demonstrated

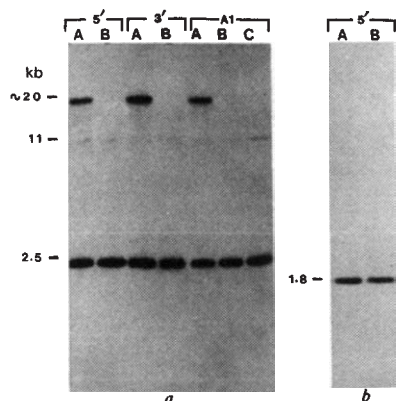


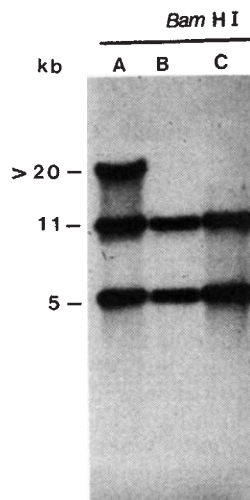
Fig. 3 Confirmation that the extra fragment hybridizing to pcBA1 contains intact most of the VSG A mRNA sequence. *a*, Hybridization of 5' and 3' fragments of pcBA1 to *Eco*RI digests of DNA from trypanosome clones expressing (A) and not expressing (B, C) VSG A, compared with hybridization of the whole cDNA. The 5' fragment contains 150 base pairs (bp) of the 900-bp cDNA sequence; the 3' fragment contains 380-bp, and includes part of the poly(A) tail. Both ends of the cDNA sequence are detected in the extra *Eco*RI fragment in clone A. *b*, Hybridization of the same 5' probe to *Pvu*II-digested DNA from clones A and B. The same *Pvu*II fragment, which contains most of the VSG A mRNA sequence, is produced from the extra copy unique to clone A and the copy present in non-expressing clone. Restriction enzyme fragments of pcBA1 were isolated by the procedure of Maxam and Gilbert¹⁶. Labelling of probes, electrophoresis and filter hybridization are described in Fig. 2 legend.

with enzyme *Pvu*II, (Fig. 3*b*). In clone A, an increase in intensity was observed in a 1.8-kb enzyme fragment, which was produced by the *Pvu*II site in the cDNA and another 1.8 kb towards the 5' end of the mRNA. Thus we suggest that the duplicated copy of the VSG A coding sequence is an intact copy of the cDNA sequence. It is reasonable to conclude that we are observing the same kind of duplication-transposition event as reported for other VSG genes.

Figure 4 shows that there are sequence differences between the basic copy and the duplicated extra copy of the clone A gene. We know from the published sequence of the clone A cDNA⁹ that the coding sequence for the A antigen is not cut by *Bam*HI. As seen in Fig. 4, the extra copy of the clone A gene was not cut, whereas the basic copy in all cell clones was cut by this enzyme. A comparison of the cDNA sequence with the basic copy sequence showed that a point mutation has occurred at base position 1,700, resulting in the loss of a *Bam*HI site in the cDNA and therefore presumably in the duplicated extra copy of clone A (see ref. 13).

In previous descriptions of this type of gene rearrangement for the VSG B genes^{2,5,6}, we suggested that the differences between our observations with this gene, and those of others

Fig. 4 Restriction enzyme site difference between the extra fragment and the basic copy of the clone A gene. pcBA1 was hybridized with *Bam*HI digests of DNA from expressing (A) and non-expressing (B, C) trypanosome clones. The probe recognizes two fragments (11 and 5 kb) in all clones and a fragment >20 kb in the expressing clone alone. When used as probes, the fragments from extreme ends of the cDNA (as in Fig. 3) show that the 11 and 5 kb fragments are respectively 5' and 3' portions of the basic copy (data not shown). This indicates the presence in the basic copy of a *Bam*HI site which is absent from both the cDNA and the additional, larger fragment unique to the expressor.



with other VSG genes, might be due to the use of different trypanosome stocks. This explanation cannot hold, as both types of gene rearrangement affect different VSG genes expressed within one sequentially derived series of trypanosome clones. It is evident that the 'expression-linked copy' model for VSG gene expression, as proposed by workers who observed extra copies of VSG genes in expressed states^{3,4,12}, does not describe the expression of the VSGs B and C in this series of trypanosome clones. Therefore, there must be some other mechanism controlling expression of these genes.

VSGs B and C are frequently expressed in relapse populations in animals infected with other ILTAR 1 clones (J. J. Doyle, personal communication), whereas VSG A is only rarely observed in relapse populations. This difference in VSG expression may be related to the different types of nuclear DNA rearrangements described here.

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Post-translational modification of tubulin dependent on organelle assembly

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Microtubules are involved in a wide variety of cellular functions¹⁻³ and are major components of many subcellular structures (for example, the centriole, mitotic spindle, cytoskeleton and flagellar apparatus). The ability of microtubules to serve in such a diversity of functions may be accounted for, at least in part, by heterogeneity in the constituent types of α - and β -tubulin subunits⁴⁻¹⁰ in different microtubules. Higher eukaryotes generally possess several tubulin genes and this may account for part of the heterogeneity of tubulin subunits¹¹⁻¹³. However, the unicellular eukaryote, *Chlamydomonas reinhardtii*, has only two α - and two β -tubulin genes¹⁴⁻¹⁶, which suggests that some of the variation in tubulin subunits seen in this organism^{5,8} may arise as a result of post-translational modification. This notion is supported by the experiments of Lefebvre *et al.*⁸ who compared flagellar tubulins with those produced by *in vitro* translation of tubulin mRNAs. Here we show that a form of α -tubulin subunit apparently confined to the *Chlamydomonas* cell body is converted, post-translationally, into a flagellar form of α -tubulin and that this modification is dependent on flagellar assembly. We discuss possible mechanisms for these modifications and their implications for the generation of unique types of microtubules having specialized functions within the cell.

When flagella are removed from *Chlamydomonas* gametes, the regeneration of a new set of flagella begins after ~10–12 min¹⁷. The initial rate of outgrowth is quite rapid (~0.5 $\mu\text{m min}^{-1}$) but diminishes exponentially as the flagella lengthen. The new flagella approach their final length by ~90 min. During flagellar outgrowth, the synthesis of tubulin, the major structural protein of the flagellum, is rapidly induced^{14,18,19}. Our studies have demonstrated that the synthesis of α - and β -tubulin subunits begins in less than 10 min after deflagellation and then rapidly increases to maximal rates in ~30–40 min. The autoradiograph shown in Fig. 1 illustrates this; it also reveals that in the SDS-polyacrylamide gels chosen for these experiments, the α -tubulin subunits produced from 10 to 90 min after flagellar excision can be resolved into two discrete bands. We have designated the upper band as α_f as it co-migrates with the predominant species of α -tubulin in the flagellum (see track 8 and also Fig. 2). The lower band co-migrates with the α -tubulin subunit species found exclusively in the cell body (see Fig. 2). Production of the α_f tubulin subunit is detectable neither during the first 10 min after flagellar removal nor beyond the time (~90 min) when flagellar outgrowth has been completed. The fact that α_f does not accumulate in the body of the cell and is produced exclusively during the period of flagellar assembly suggests that a portion of the newly synthesized α -tubulin subunits is converted to a modified form in a process associated with flagellar outgrowth. The results of the following experiments support this conclusion.

If mRNA is isolated from cells 45 min after deflagellation, when the production of α_f is at its peak (Fig. 1), and then translated in the wheat germ *in vitro* translation system¹⁸, an α -tubulin polypeptide is produced which co-migrates with the α -tubulin of the cell body (Fig. 2a, lanes 4 and 7). There is no detectable synthesis of a polypeptide having the same electrophoretic properties as α_f (Fig. 2, lane 4 compared with lanes 3 and 5). These results suggest that either a mRNA encoding α_f exists but is not translatable *in vitro* or all cellular α -tubulin mRNAs are translated faithfully *in vitro* and produce α -tubulin which is identical (or almost identical) to the α -tubulin synthesized and stored in the cell body. If the latter is the case, then α_f could only arise as a result of post-translational modification. This interpretation is consistent with that of Lefebvre *et al.*⁸ who performed similar *in vitro* experiments using the rabbit reticulocyte translation system. Nevertheless, the possibility still exists that a putative α_f mRNA does exist and that neither the wheat germ translation system nor the reticulocyte *in vitro* system is capable of translating it into a mature polypeptide. To establish that α_f is indeed the product of post-translational modification, we performed two additional sets of experiments.

In the first, we took advantage of the fact that tubulin induction following deflagellation is not dependent on subsequent flagellar outgrowth¹⁷. Flagellar regeneration was blocked in this experiment by treating deflagellated cells with colchicine. A comparison of tubulin species produced by deflagellated cells in the absence (Fig. 2b, lane 1) and presence (lane 2) of colchicine revealed that the α_f subunit was produced by the non-treated cells but not by a sample of the same cells treated with colchicine immediately after flagellar excision. As both samples were labelled for the same time period after deflagellation, these results also indicate that the appearance of α_f is dependent on flagellar outgrowth. (To ensure that the load size of protein in any of the samples did not influence the relative migrations of α - and α_f -tubulin subunits, radioactive proteins from flagella or cell bodies were combined with appropriate samples of non-radioactive proteins from the same sources as described in Fig. 2 legend, and run in Fig. 2b, lanes 3, 6 and 7.)

In the second experiment, cells were deflagellated and allowed to produce tubulin subunits for 120 min (Fig. 2c, lane 1) during which time flagella were regenerated (lane 4) and reserves of radioactively labelled tubulin accumulated in the cytoplasm (lane 6). At 120 min, cycloheximide was added at a concentration of 10 $\mu\text{g ml}^{-1}$ which blocks essentially all protein synthesis on 80S ribosomes in *Chlamydomonas* (ref. 17; Fig.

2c, lane 3). The cells were then deflagellated²⁰, rapidly chilled, and centrifuged to separate out the labelled flagella. The pelleted cell bodies, which were free of contaminating flagella as judged by microscopic examination and by the lack of α_f -tubulin subunits (lane 6), were resuspended in fresh medium and allowed to regenerate flagella for 1 h. As expected^{18,21}, the cytoplasmic reserves of flagellar proteins allowed the regeneration of flagella that were on average 4–5 μm long (compared with 12–15 μm for full-length flagella). These short flagella were removed and separated from the cell bodies. Examination of the α -tubulin in the flagella regenerated in the presence of cycloheximide revealed that α_f -tubulin subunits were produced even in the absence of new tubulin synthesis (Fig. 2c, lane 5). None of the radioactively labelled α -tubulin that was converted to α_f during regeneration in the presence of cycloheximide was found in the body of the cell; α_f was found only in the flagella.

Taken together, these data provide strong evidence that the major α -tubulin subunit of the flagellum is the product of post-translational modification and, more importantly, that this modification is coupled to flagellar assembly. Two mechanisms can be envisaged to account for the close coupling of post-translational modification of α -tubulin and flagellar outgrowth. In the first, α -tubulin is modified in the cytoplasm only during periods of flagellar elongation and, once modified, is rapidly and quantitatively transported into the growing flagellum. The second, and perhaps more attractive hypothesis, is that

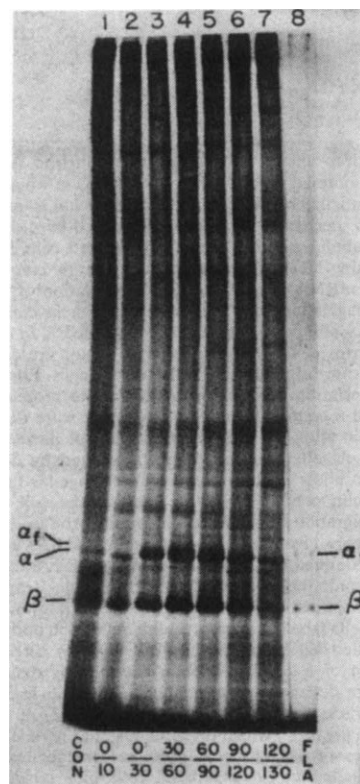


Fig. 1 Tubulin induction following flagellar excision in *C. reinhardtii*. Autoradiograph of a SDS-polyacrylamide gel containing $\text{H}_2^{35}\text{SO}_4$ -labelled proteins produced at various times before and after flagellar excision. Preparation of gametic cells by transfer of vegetative cells to nitrogen-deficient media and deflagellation of these cells by pH shock were performed as described previously¹⁷. Gametic cells were pulsed with $\text{H}_2^{35}\text{SO}_4$ before deflagellation (CON) and for the 10- or 30-min periods after flagellar removal shown below each track (for example, 30/60 denotes labelling from 30 to 60 min after deflagellation). Labelling conditions were as previously described¹⁷ and included the use of chloramphenicol (250 $\mu\text{g ml}^{-1}$) to prevent the synthesis of chloroplast proteins. Samples having $\sim 2 \times 10^6$ c.p.m. were loaded on to each track of the 15 cm-long 8% polyacrylamide gel and the proteins separated by their differential electrophoretic mobilities¹⁸. α Designates the α -tubulin subunit which migrates as a protein with an apparent molecular weight of 56,000; α_f , the predominant α -tubulin subunit of the flagellum; β , the β -tubulin subunit of molecular weight 53,000. Track 8 contains radioactively labelled flagellar markers (2×10^5 c.p.m.).

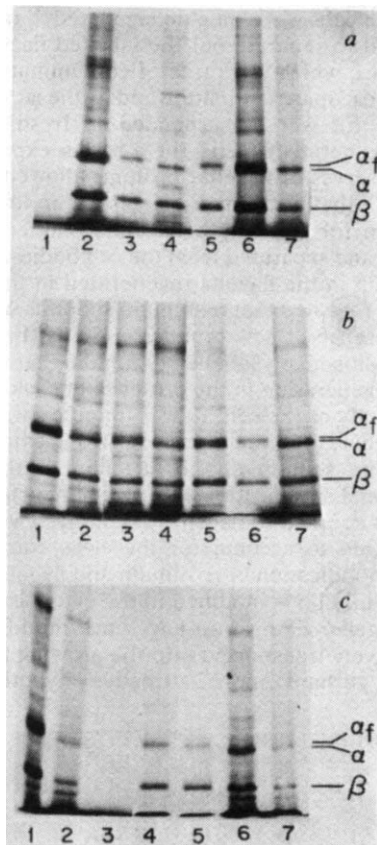


Fig. 2 Analysis of α - and α_f -tubulin subunit production. Autoradiographs of 8% polyacrylamide gels (containing 0.1% SDS) in which samples from cells and flagella labelled *in vivo* and samples of *in vitro* translation products ($\sim 1\text{--}2 \times 10^5$ c.p.m. per track) have been separated by electrophoresis. *a*, Comparison of tubulin subunits from deflagellated cells labelled *in vivo* with ^{35}S -methionine-labelled products from the *in vitro* translation of *Chlamydomonas* mRNA. Procedures for the isolation of polyadenylated mRNA from deflagellated cells and its translation *in vitro* in the wheat germ translation system were as previously described¹⁸. *In vitro* translation reactions were performed in the absence (track 1) or presence (track 4) of mRNA isolated from fully-induced deflagellated cells. For comparison of these *in vitro* translation products with radioactive proteins produced *in vivo*, tracks 2 and 6 contain samples of cells that were deflagellated and labelled for 60 min with ^{35}S - H_2SO_4 . An aliquot of these same cells was deflagellated and flagella and cell bodies separated by density gradient centrifugation²⁰ to allow further comparison of flagellar tubulin (tracks 3 and 5) with tubulin contained in the body of the cell (track 7). The electrophoretic migration of the tubulin subunits produced *in vitro* and *in vivo* was not influenced by differences in protein load sizes as demonstrated by the following combinations of components: track 2, a mixture of the *in vitro* translation components of track 1 with proteins from deflagellated cells labelled *in vivo*; track 3, a mixture of the *in vitro* translation components of track 1 and flagella labelled *in vivo*; 5, unlabelled cell bodies and labelled flagella; 7, unlabelled flagella and labelled cell bodies. *b*, Effects of colchicine on the production of α - and α_f -tubulin subunits by deflagellated cells. Gametic cells were deflagellated and labelled with ^{35}S - H_2SO_4 for 60 min in the absence (tracks 1 and 5) or presence (track 2) of colchicine at a concentration (1.5 mg ml^{-1}) which prevents flagellar regeneration. Control and marker samples were as follows: 4, proteins from labelled, non-deflagellated cells; 3, labelled non-deflagellated cells plus labelled flagella; 6, labelled flagella plus unlabelled cell bodies; 7, labelled cell bodies plus unlabelled flagella. *c*, Conversion of pre-labelled α -tubulin subunits into α_f -subunits during flagellar regeneration in the presence of cycloheximide, a protein synthesis inhibitor. Gametic cells were deflagellated and labelled with ^{35}S - H_2SO_4 for 120 min (track 1). Non-deflagellated cells were similarly labelled in the absence (track 2) or presence (track 3) of cycloheximide ($10\text{ }\mu\text{g ml}^{-1}$). At the end of the labelling period, cycloheximide was added and a sample of the cells which had regenerated flagella were again deflagellated in order to allow separation of labelled flagella (track 4) from labelled cell bodies (track 6) by density gradient centrifugation. The pelleted cell bodies were resuspended in fresh medium containing cycloheximide and allowed to regenerate new flagella for 60 min in the absence of protein synthesis, then the cells were once more deflagellated to separate flagella (track 5) and cell bodies (track 7). In this case, after the cell bodies and flagella had been separated by centrifugation, the flagellar fraction was centrifuged at 30,000 r.p.m. for 45 min in a Beckman SW 40.1 rotor (as opposed to the usual centrifugation at 16,000 r.p.m. for 30 min in a Sorvall SS 34 rotor) to ensure the complete recovery of the short flagella regenerated in the presence of cycloheximide.

unmodified tubulin subunits move into the flagellum and are modified at or near the site of active microtubule assembly. In the latter case, localization of the enzymes responsible for tubulin modification might be accomplished by their binding to structures closely associated with the growing tips of the axonemal microtubules (for example, the distal filaments described by Dentler²²).

The present and earlier^{6,23} observations of flagellar-specific tubulins suggest that, in general, the ability of microtubules to perform a specific function in a particular subcellular structure may be influenced by the types of tubulin subunits they contain. Differences in the tubulin composition of microtubules can, in turn, be influenced by either genetic variability between different tubulin genes in the cell or post-translational modification of tubulin subunits. The presence of multiple tubulin genes in several higher organisms^{11,12} indicates at least the potential to generate subunit diversity at the genetic level. However, two observations raise some question as to the role of genetic variability in the production of different classes of microtubules within a single cell. First, in *Aspergillus*, a heat-sensitive mutation in a single β -tubulin gene²⁴ seems to have pleiotropic effects on several microtubule-mediated functions—suggesting that tubulin produced from a single gene can be incorporated into most, if not all, of the microtubules of the cell. Secondly, in *Drosophila*, a mutation has been found in the structural gene for a β -tubulin that is expressed only in testis tissue⁷. The variant tubulin produced from this 'specialized' tubulin gene is apparently not confined to a particular type of microtubule, but rather has wide ranging detrimental effects on various microtubule-containing structures within the cell. Therefore, if the existence of organelle-specific tubulin subtypes is widespread, the production of these subtypes may well have to depend on the kinds of post-translational modifications reported here for the α_f -tubulin subunit of the *Chlamydomonas* flagellum.

Finally, one hypothesis that may be drawn from our results is that the enzymes required for flagellar α -tubulin modification may be located close to the site of axonemal microtubule assembly. In a broader context, this hypothesis suggests that the enzymes which modify tubulin subunits and thereby tailor microtubules to perform the specialized functions of a particular organelle may not be scattered throughout the cytoplasm but may be in close and perhaps exclusive association with the microtubules of that organelle.

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BOOK REVIEWS

A new nuclear holocaust

John Maddox

IN 1962, Mrs Rachel Carson discovered the harmful effects of pesticides in the pages of the *New Yorker*, and life has not been the same since for the chemical manufacturers. Earlier this year, Mr Jonathan Schell used the same vehicle to announce his personal discovery that nuclear weapons also have harmful effects, and in the process helped to fuel the influential campaign in the United States for a "nuclear freeze". Like *Silent Spring*, *The Fate of the Earth* is a republished collection of the magazine articles.

The result is compulsive reading but not necessarily a compelling book. The first half is the simpler and the more arresting, written as it is with restrained passion, suppressed rage perhaps. Yet Schell is not an out-and-out scaremonger but rather a cautious one. His "nuclear predicament" is his way of referring to people's indifference to the threat embodied in the nuclear arsenals — "the world has declined, on the whole, to think about them very much". Properly and persistently he reminds his readers that uncertainties must abound in attempts such as his to show what would happen in an all-out nuclear attack on the United States. Would more people be killed by prompt radiation from an airburst weapon than from fire or blast? Only time, says Schell, could tell.

But how can it be possible to write vividly about a topic, the destruction that might be caused by a nuclear attack, when thousands on thousands of words have already been cast in type? Schell succeeds for two reasons, one less creditable than the other.

The meaner trick is the assumption of discovery:

We have lived in the shadow of nuclear arms for thirty years, so it does not seem too soon for us to familiarize ourselves with them . . .

or

a description of a full-scale holocaust seems to be made necessary by the simple but basic rule that in order to discuss something one should first know what it is.

There follows a list of the technical reports that Schell has referred to, but there is no acknowledgement of other writers in the same genre — Robert Junk with his *Brighter than a Thousand Suns*, for example. One's first reaction is resentment: why should Szilard, for example, rate a mention in Schell's account only as one "who called on a number of

The Fate of the Earth. By Jonathan Schell. Pp.244. UK hbk ISBN 0-224-02064-1; UK pbk ISBN 0-330-26915-1; US ISBN 0-394-52559-0. (Jonathan Cape/ Picador/Knopf: 1982.) Hbk £7.95, \$11.95; pbk £1.95.

colleagues to keep the discovery [of fission, not then of the bomb] secret from the Germans"? Was he not the Manhattan Project man who wore his conscience most conspicuously on his sleeve? But charitably, one quickly comes to terms with Schell's assumption. To discover the prospective awfulness of the holocaust is more likely to catch the attention than to recount what has been told already. Schell is in any case properly diffident about his knowledge of distant historical events (using the phrase "it is reported" to describe what happened during the Cuban missile crisis of 1962); and then, perhaps, each generation that lives with the bomb needs its own prophet.

Schell's second trick, his literary skill, gives him a good chance of being that. His account of what the holocaust might be like is original, almost fresh. Fission, as fusion, is the exception that proves Einstein's rule that mass can be converted into energy. The novelty in fission is that the strong nuclear force was for the first time manipulated by people. One gram of uranium destroyed Hiroshima, but the bomb weighed four tonnes.

The literary rehearsal of the holocaust takes off effortlessly from this point. Schell says that in "this unique endeavour", "foresight is asked to perform a task usually reserved for hindsight". He is only a reluctant futurologist. He takes comfort from the circular slide-rule distributed with copies of Glasstone and Dolan's book *The Effects of Nuclear Weapons* to ground his calculations in someone else's reality, and he does his best to follow the *New Yorker's* precept that the whole world has an absorbing interest in what happens in Manhattan by exploding his mercifully hypothetical one-megaton weapon above the Empire State Building; all the buildings between Battery Park and 125th Street would be knocked down, the fireball would set the city alight. "From Greenwich Village up to Central Park, the heat would be great enough to melt metal and glass."

Schell, of course, is telling the truth. He is probably also right in his guess of what would happen if the existing stock of Soviet nuclear warheads were fired at a representative set of targets throughout the

United States. Communications systems would be put out of action. Most cities not flattened would be burning. The few survivors would be either beastly or indifferent to each other, and hampered in their efforts to restart some kind of primitive society by their load of genetic defects and consequent somatic transformation. The critical question, for Schell, is whether the result would be to extinguish the human species or to make life on the Earth impossible, perhaps by the removal of the ozone cover, for all except a few plants and radiation-resistant insects. He acknowledges that the outcome is uncertain, but although there is "all the difference in the world" between the chance and the certainty of extinction, "morally they are the same".

Peroratively, half-way through his book, Schell remarks:

In weighing the fate of the earth and, with it, our own fate, we stand before a mystery, and in tampering with the earth we tamper with a mystery. We are in deep ignorance. Our ignorance should dispose us to wonder, our wonder should make us humble, our humility should inspire us to reverence and caution, and our reverence and caution should lead us to act without delay to withdraw the threat we now pose to the earth and to ourselves.

This purple passage is the climax to the book. Tract though it may be, it is a well-intentioned tract. Its naivety can be forgiven as innocence. Its neglect of the agonies that have exercised people of all kinds since 1938 is less easily forgiven, but Mr Schell has an important point to make.

At this high point in his book, with only half the pages turned, readers are bound to wonder what can come next. An account, probably ironical, of the attempts that people have been making over the years to negotiate arms control agreements? A discussion of the danger of nuclear proliferation, and of the wayward policies of the nuclear suppliers in this regard? The answer, it turns out, is nothing like that but a chapter called "The Second Death", a kind of essay on the theme that even in strictly personal terms there is something worse than being killed — the whole species might be eliminated.

Nobody will quarrel with the conclusion. Did not even St Augustine, for whom the objective of every human being was to arrive safely in Heaven, acknowledge that even quite ordinary people could be fixated on the terrestrial future by their regard for their children and their children's children?

But Schell skates on thin ice in getting there.

Thus he asserts that scientific knowledge is different from other knowledge in that it cannot be forgotten. Really? And that

a disturbing corollary of the scientists' inability even to foresee the path of science, to say nothing of determining it, is that while science is without doubt the most powerful revolutionary force in the world, no-one directs that force.

But Schell's real objective is to contrast and compare the notions of death and extinction, one personal, the other for the species. He rightly and evocatively finds a parallel between the extinction of wagon-loads of people in the concentration camps and the feared extinction of whole species. It is an ethical goal, he says, to seek to avoid extinction. Yet living as we do on top of a nuclear stockpile, we are "living with a lie".

The core of Schell's final essay is a comment on the doctrine of deterrence, a popular target these days. If the objective is to build a weapons system so powerful that the destruction of an adversary can be assured, how can success be made convincing without a demonstration? But detente is no good either: look at the way the Soviets made "crimes against detente" punishable, and how President Nixon pleaded exemption from the laws of the United States for the sake of a peaceful world. The real bugbear, Schell says, is sovereignty (but his book was written before the Falkland Islands conflict).

... just as those who favour the deterrence policy ... must in all honesty admit that their scheme contemplates the extinction of man in the name of protecting national sovereignty, so must those who favour complete nuclear and

conventional disarmament, as I do, admit that their recommendation is inconsistent with national sovereignty . . .

Read as a piece (and it does not take long), Mr Schell's book is thus an extraordinary let-down. "I have not sought to define a political solution to the nuclear predicament" but "I have left to others those awesome urgent tasks . . .". There's generosity for you; some other author, another four-part series in the *New Yorker*, perhaps?

What Mr Schell has forgotten (apart from the precursors whom he fails to mention) is that the simplest explanation of his opening conundrum — why are we

mostly so indifferent? — is not a failure to imagine what nuclear war would do to us but a failure to devise political procedures for its sure avoidance. People have seen the difficulties and have sometimes lost heart. Mr Schell's recipe, if taken seriously, can only further depress them, for in his eloquent way he is saying that the problem would be manageable if only the world were an entirely different place. For a book that makes so much of people's ethical responsibilities, that is not merely a disappointing conclusion but an irresponsibly airy recipe for conduct. □

John Maddox is Editor of Nature.

Affinity for industry and biomedicine

P.G.H. Byfield

Affinity Chromatography and Related Techniques: Theoretical Aspects/Industrial and Biomedical Applications. Analytical Chemistry Symposia Series, Volume 9. Edited by T.C.J. Gribnau, J. Visser and R.J.F. Nivard. Pp.584. ISBN 0-444-42031-2. (Elsevier Scientific: 1982.) Dfl.195, \$83.

AFFINITY chromatography has marched out of the research laboratory and into the world. That was to be the message of the symposium held in June of last year at Veldhoven, of which this book is a record, and the organizers emphasized it by bringing together similar numbers of participants from industry and academia. Meetings based on the discussion of a technique run the risk of being boring since the wide range of possible applications may contain only little of interest to an individual. The organizers overcame this by concentrating the contributions around biomedical applications and relevant industrial processes. This theme penetrated also into the contributions on theoretical considerations and developments in supports and chemistry; many of these were based on biomedical or industrial needs.

By far the most exciting current application of affinity chromatography is indeed to the biomedical field. Even the ability to simplify the isolation of useful compounds from biological sources will open up new possibilities in patient treatment and management. Several contributions describe the commercial production of proteins from human plasma by this technique, others indicate the way to future products, while one author laments that only lack of knowledge of their function inhibits the isolation of more plasma constituents — perhaps the physiologists will take up this challenge. Exploiting the high specificity of antibodies in therapy, as reagents and as affinity ligands, has often been hindered by difficulties in their isolation due to the high interaction energies between antigen and antibody.

The successes described here in the fractionation of antisera and in the purification of vaccines will help many, particularly when combined with the practical conclusions in the theoretical paper by van Oss.

Some disquiet was expressed at the meeting that ligand leakage might contaminate products intended for clinical use with hazardous compounds, especially where organic dyes are the ligands. These problems should be easy to overcome. After all, what is affinity chromatography about if not to remove small quantities of a substance from large quantities of other material?

One forward-looking section of three papers considered the use of affinity systems directly in therapy, for the delivery of drugs to specific sites *in vivo* and for the extracorporeal treatment of blood to remove toxins, drugs and so on. Although there is a long way to go, these approaches to more specific treatment of disease are surely to be encouraged.

It is rare to read of an affinity chromatography system that is not based on cyanogen bromide-activated agarose, yet most meetings include sections on new supports and coupling methods. Why is this? One answer was evident at Veldhoven. Both large-scale work and the new high-pressure liquid affinity chromatography systems need robust supports such as the glass, silica and polymers described by several speakers.

Many good posters were shown at the meeting and it is a pity that, tantalizingly, only the titles, not their abstracts, have been published in the book. Nonetheless those interested in using the technique rather than in studying the phenomenon will find much of value in this volume; even the theoretical section contains advice which will be of much practical use. □

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Journals' review issue 1982

On October 7th *Nature* will publish a second review supplement devoted to science journals. Last year's supplement, covering journals first published between January 1978 and May 1980, appeared in *Nature* 293, 341-369; see p.343 of that issue for details.

Criteria for inclusion of a journal in the 1982 issue are that:

- the first number appeared, or the journal was re-titled, between June 1980 and May 1981;
- it is published at least three times a year;
- the main language used is English.

Broadly, periodicals of professional interest to scientists will be considered for review, with the exception of abstracts' journals.

In addition it is hoped to cover publicly available newsletters, first published between January 1978 and May 1981, in that issue.

Publishers and societies are invited to submit four sample issues of periodicals satisfying the above criteria, including the first and most recent numbers, to the Review Editor, *Nature*, 4 Little Essex St, London WC2R 3LF, England (London 836 6633 ext 2570) as soon as possible.

Uneven depths in marine geology

Philip Weaver & Lindsay Parson

The Oceanic Lithosphere. Edited by Cesare Emiliani. Pp.1,738. ISBN 0-471-02870-3. (Wiley: 1982). £110, \$195.

SINCE the inception of the Deep Sea Drilling Project, which has played a major role in providing data from previously unsampled horizons in both sediments and the basaltic basement, marine geology has emerged from infancy and into an exciting period of discovery. More recent work using manned submersibles has proved invaluable for the precision sampling of specific areas of interest. Investigations have not been restricted to these data, however, and bottom sampling and geophysical work outside the DSDP are providing the basis for new studies, such as the extensive use of gravity and piston core sampling in the investigation of Quaternary sediments (for example in the CLIMAP project).

The Oceanic Lithosphere, the seventh volume of *The Sea* series, attempts to bring together some of the new discoveries and to provide reviews of the major fields of research. Its scope is therefore enormous, and no one book could hope to cover all aspects fully. The subject matter of the 35 chapters falls naturally into two sections, the first dealing with upper mantle and crust, the second with the sedimentary record.

It is noteworthy that a number of the review articles — such as those on heat probe and on magnetism, and on the composition and evolution of the ocean crust — constitute works of equal, if not greater value to researchers of a general geological or geophysical bent seeking broad appraisals of the ocean basins than to the specialist oceanographers to whom this book claims to be directed. In contrast, or perhaps as a balance, a number of detailed discussions — such as those considering helium isotope work and radiogenic gas retention — will be of far more limited appeal, and may have been included at the expense of swelling an already impressively over-large tome.

Nevertheless, valuable contributions are made in the form of studies of ophiolite suites and their role in the understanding of the evolution of present-day oceanic crust. Petrological analyses of oceanic basement are well represented, and in particular the review by Fox and Stroup provides a comprehensive synthesis of work on oceanic crust with its discussions of composition, seismic structure, heat flow results and magnetism.

The section on marine sediments covers a range of topics from sediment composition and origin to the evolution of ocean basins. Apart from red clays, most oceanic sediments are biogenic in nature, and analysis of their microfaunas enables us to understand past climates and ocean circu-

lation patterns, and to identify changes in the normal sedimentary regime such as hiatuses, anoxic intervals and periods of mass extinctions. *The Oceanic Lithosphere* necessarily devotes several chapters to reviews of the various microfossil groups, providing comprehensive summaries of the particular group, their present-day and past distributions, and their value to oceanography. These are followed by chapters reviewing the deep-sea record and the dynamics of the Earth's sediment system as a whole.

One of the areas of particular interest at the moment is the study of sedimentary processes. Identification of sediment drifts, submarine slides and erosional channels, and the mapping of turbidite flows

shows us that the ocean floor is not necessarily stable. Regrettably, this area of research is not covered in the book and readers seeking an overview of the subject could be left with the misconception that the oceanic sedimentary record consists of a layered sequence of undisturbed sediments.

Taken as a whole the volume offers a somewhat patchy review of the oceanic lithosphere. It would have been more logical to produce it as two separate parts as with other volumes in this series, and perhaps to add a few more topics to each part. Largely as a result of the amalgamation of these two parts, *The Oceanic Lithosphere* appears destined for the reference collection kept on a sturdy shelf. □

Philip Weaver and Lindsay Parson are at the Institute of Oceanographic Sciences, Wormley, Surrey.

All round the world of algae

Brian Whitton

The Ecology of Algae. By F.E. Round. Pp.653. ISBN 0-521-22583-3. (Cambridge University Press: 1981.) £60, \$130.

PROFESSOR Round has presented us with the type of book which is becoming rarer and rarer, a major monograph written by just one person. Algae are a decidedly heterogeneous group of organisms, so the author has faced up to a challenge which would daunt most editors of multi-author symposium proceedings. *The Ecology of Algae* has well over two thousand references integrated into the text, so it approaches in scope such famous works as F.E. Fritsch's *Structure and Reproduction of the Algae* (Cambridge University Press; 1935, 1945) or one of the volumes of G.E. Hutchinson's *A Treatise in Limnology*, published by Wiley. The question, then, is just how well has the author succeeded in covering this vast topic.

Professor Round has himself published papers on freshwater and marine environments, and has travelled and researched in many countries. The book obviously benefits from this, as both types of environment are given equally sympathetic treatment and examples are chosen on a world-wide basis. There is only one specifically marine chapter, on coral reefs. Throughout the rest of the book the algal ecology of freshwater, subaerial and marine environments is brought together, usually in adjacent sections, but sometimes in a single integrated account. This approach is often very successful in showing similarities between different environments, particularly among the phytobenthos communities which were the subject of much of the author's earlier research. As far as I can judge, most topics are dealt with thoroughly and competently, but in

comparison with the space given to the behaviour of algae, treatment of the environment is brief. Information on nutrient cycles is particularly sparse and rather uncritical.

The text steers a reasonable course between the summary of numerous case studies and synthesis; there is none of the nonsense associated with the glib generalizations of several modern algae textbooks. A great deal of information is packed into each section, so the text often requires hard concentration; many readers will probably use the book more as a means of finding their way into the literature than as one from which whole chapters are read in a single sitting. I enjoyed especially Round's own comments on the state of particular areas of research. Such asides can be decidedly annoying, but Round's often show considerable insight and are so sensible that I found them most helpful.

The book is well produced, with numerous figures, useful summaries at the end of each chapter, few printing errors and accurate references. An appendix summarizes the current state of algal systematics, though (to quibble) I would disagree with the inclusion of the Prochlorophyta as a phylum. The main text reviews a large body of literature with a sweep that few, if any, other phycologists in the world could attempt. Research on ecology dates much more slowly than that on physiology and biochemistry, so *The Ecology of Algae* is likely to remain an important text for at least the next decade. I hope librarians will find the money to buy this book, whatever the state of their finances. □

Brian Whitton is Reader in Botany at the University of Durham.

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Ape reproduction: science and ethics

Alison Jolly

Reproductive Biology of the Great Apes: Comparative and Biomedical Perspectives. Edited by Charles E. Graham. Pp.437. ISBN 0-12-295020-8. (Academic: 1981.) \$52.50, £34.80.

THIS is an unusual book: it achieves just what it sets out to do. It is a handbook that summarizes existing knowledge of ape reproduction, and in the process also raises new ideas which will shape future research. And yet, for those few people who read the entire volume, the clearest impression is not the zoological synthesis the various authors achieve, but an ethical chasm which divides them.

To be sure, the synthesis is impressive: studies of ape reproductive tracts, secretions, hormonal control, pregnancy and birth, endocrine development, sexual behaviour in laboratory or field, as well as conditions of caging and handling for different studies, are brought together and analysed thoroughly. The book shows that the field observations do correlate with laboratory studies of anatomy and physiology; one can indeed illuminate the other. This volume will thus be an essential reference source.

But how do these ape studies apply to human beings? Here we reach the gulf between the indoor and the outdoor scientists. Each of the physiological articles ends by saying that ape reproductive parameters are much closer to those of human beings than to rhesus monkeys'. Each then concludes that we must conserve the great apes for use in medical experiments unethical with human beings, though most add that both ethics and commercial good sense mean limiting damage to such 'models' to a minimum.

The field workers, instead, want apes conserved for their own sake and for the scientific and aesthetic pleasure they give us. Perhaps they even share my distaste for calling a living animal a 'model', which implies that it exists only as a human surrogate. They certainly emphasize conservation of wild habitat with its mothers that date five years on one infant before bearing the next, instead of 'rational' rearing in nurseries. Battery ape production means removing infants from the mother at birth, if destined for experimental use, so that mothers skip lactational amenorrhea and get on with the next pregnancy. At present, a breeding nucleus of infants must be left for two years with the mother before removal if they are to become emotionally capable of adult sexual intercourse. Later perfection of artificial insemination might remove any 'need' for either ape sex or ape motherhood.

I exaggerate, but only slightly. Understanding ape reproduction is desperately important to maintain the captive populations: 1,000 chimpanzees in the United

States; far fewer gorillas and orang utans. In Great Britain and Ireland, as of January 1980, zoos held 167 chimpanzees, 63 gorillas and 61 orang utans, few of them breeding adults. We may argue cynically that we shall not pay to support these creatures and their remaining wild cousins unless we selfishly think of them as 'models', or we may frankly argue that they should be preserved for other reasons. Of the physiologists who have contributed to this book, only R.V. Short has the courage of his logic as well as his convictions to conclude:

Humans not only are more available, but also infinitely more cooperative than apes; human reproductive biology is more likely to be pioneered in the human, and it is seldom that the basic facts will first be revealed in an ape, and subsequently extrapolated to man . . . If the only great apes to survive in the 21st century are to be found in zoological gardens and laboratories, we will have lost forever many of the vital behavioral clues which explain why these animals are built the way they are. The great lesson of evolution is that form reflects function. Bequeathed only a caged, or worse still, a stuffed gorilla, we would have no way of determining the adaptive significance of any of its anatomical features . . . if we can begin to correlate form with function in our closest relatives, then by analogy we can gain new insights into the selective forces that have made humans what they are today.

Alison Jolly is a Guest Investigator at The Rockefeller University, New York.

Knowing one's place

R. H. S. Carpenter

Human Visual Orientation. By Ian P. Howard. Pp.697. ISBN 0-471-27946-3. (Wiley: 1982.) £25, \$59.95.

OUR senses provide us with two distinct kinds of information: *what*, and *where*. Neurophysiologists, beguiled by the complexities of *what*, have tended to neglect *where*; yet the location of stimuli is far from being the simple matter one might at first suppose.

Objects can only be localized relative to the organ that senses them; if this is itself moveable, then we need to know not just where the object is relative to the sense organ, but also where the sense organ is relative to the body. In the case of vision, we have a highly mobile eye mounted on a freely turning head, attached to a flexible body which can be moved about with the feet. Add to this the large number of degrees of freedom enjoyed by our arms and hands, and it can be seen that even an

action as simple as picking up a pencil demands the complex processing of information not just from our eyes, but from sense organs in our muscles, skin and joints, from the vestibular receptors that tell us about the motion and attitude of our head relative to gravity, and from the stored expectations that we have of the way the world is structured.

To a very large extent, this integration of various kinds of information must be learnt: unlike a surveyor's theodolite, our senses do not come to us ready-calibrated in degrees and minutes of arc, but must be scaled by our own brain through its experience of the way in which one kind of sensation tends to be correlated with another. If we wear prisms that deviate our sight to one side, though at first we reach out for visual objects at the wrong place, after only a few trials our brain adjusts itself to the new situation and no errors are made. This aspect of sensory location has stimulated a great deal of interest over the past few years, for it enables one to make attractively quantitative measurements of the learning processes involved. The advantage of studying sensorimotor coordination rather than perception is that we then work with a system with a clearly defined output as well as input; in the latter case there must always be some doubt as to the nature of whatever it is that is doing the perceiving.

Behind a deceptively modest title, *Human Visual Orientation* hides a wealth of detailed and remarkably up-to-date information about nearly all aspects of this very wide subject. Particularly welcome, in a field where slipshod terminology can easily lead to tangled confusion (how, for example, does one rigorously define the direction of gaze?), are the author's succinct definitions of technical terms, and his clear and intelligent reviews of salient facts in an area in which many important observations date back to the nineteenth century. Many topics which the title of the book might not lead one to expect — the neurophysiology of eye movements, for one — are summarized with equal clarity and expertise.

In other respects, however, the author has perhaps been too modest in his aims: his explicit omission of motion perception and other dynamic aspects of orientation, and perhaps especially his suspicion of model-building and his reluctance to synthesize and integrate on more than a moderate scale, may leave some readers unsatisfied. But this does not detract significantly from what is otherwise a first-rate book, a richly-veined mine of information that will undoubtedly become a standard work of reference and provide much to ponder upon for many years. □

Roger Carpenter is a Lecturer in Physiology at the University of Cambridge. He is author of Neurophysiology, shortly to be published by Edward Arnold, and of Movements of the Eyes (Pion, 1977).

To whom does the United States belong?

Lynton K. Caldwell

The Land Use Policy Debate in the United States. Edited by Judith I. de Neufville. Pp.269. ISBN 0-306-40718-3. (Plenum: 1981.) \$29.50, £18.59.

TO UNDERSTAND the land use policy debate in the United States one must first recollect its historical context. During the colonial era, and for decades thereafter, settlers were drawn to the New World by the prospect of free land unburdened by the feudal legacy of taxes and servitudes. And whereas general English common law regarding ownership and tenancy of land was brought to North America, specific restrictions were left behind. Moreover the early years of colonization occurred at a time when English landowners were shaking off feudal exactions. In any event, controls by government were largely unenforceable on the frontiers of settlement. Then, as public authority and jurisdiction were extended to unappropriated lands, the guiding object of public policy was to move ownership as rapidly as possible into private hands.

For a century after independence, speculation in the purchase and sale of public lands was one of the principal roads to wealth in the United States. And the most respected figures in public life, not excepting George Washington himself, did not hesitate to engage in it. The privatization of land was carried further in North America than perhaps in any other country in the modern world. Attempts to impose even minimal controls on the uses

of land have not come easily. In 1970 the Senate of the United States considered a National Land Use Planning Act, which was intended to encourage states and localities to adopt forms of democratic planning that would avoid at least the worst conflicts and demonstrable misuses of land. Endorsed by President Nixon, this measure passed the Senate on three occasions but never reached the floor of the House of Representatives, being blocked by a combination of land speculators, developers, farmers and ordinary citizens who feared that the government was about to take away the right to do as they pleased with the land that they owned.

Against this background, one may better understand the 20 essays that make up this book. They evolved out of a colloquium held in 1977 under the sponsorship of the Lincoln Institute of Land Policy at Cambridge, Massachusetts. In view of the long history of frustration in land use planning in the United States, it was appropriate that the focus of the colloquium was on latent values in land use policy. It should be apparent that a significant measure of change in popular understanding and attitude will have to occur before rational land use policy will become effective in the United States.

The scope of the colloquium, and the book, includes considerations of land use as affected by economic, social and environmental policy, the role of markets, the law and public participation. Finally, the authors raise the possibility of a new

The geologist's wife

TO HER HUSBAND SETTING OFF
UPON AN EXCURSION

Adieu then, my dear, to the Highlands you go,
Geology calls you, you must not say no;
Alone in your absence I cannot but mourn,
And yet it were selfish to wish your return.

No, come not until you have searched through the gneiss,
And marked all the smoothings produced by the ice;
O'er granite-filled chinks felt Huttonian joy,
And measured the parallel roads of Glenroy.

And if, in your wanderings, you chance to be led
To Ross-shire or Moray, to see the Old Red,
Oh still, as its mail-covered fishes you view,
Remember the colour is love's proper hue.

Such being your feelings, I'll care not although
You're gone from my side — for a fortnight or so;
But know, if much longer you leave me alone,
You may find, coming back you have two wives of stone!

These verses, four of the eight in *The Geologist's Wife* — written by the anonymous but evidently love-lorn "Susan" in 1847 — are taken from *The Poetry of Geology*, recently published by George Allen & Unwin. The anthology is edited by Robert M. Hazen and costs £4.95, \$9.95.

land ethic and identify a series of problems relating to political control of land use. The editor of this volume, who is a member of the Department of City and Regional Planning of the University of California at Berkeley, pulls together the principal themes and viewpoints of the authors in a final essay.

Four general policy positions emerge in the discussions, although not all of the viewpoints are shared by all authors. The first is that the test of good land use policy is the procedure by which it was adopted. This opinion divides between those who emphasize participatory democracy and those who advocate market processes. The second position is that a policy is good if it truly represents the public interest. This view of "goodness" depends less upon what people think than upon ostensibly objective assessments of the implications — economic, ecological and ethical — of particular land use decisions. A third position is that a good land use policy is one that is allied with technological or problem-solving criteria. From this viewpoint land use capability, as assessed by technological and economic possibilities, is the significant factor in determining the land's best and highest use. Fourth, and final, is the notion that good land use policy must be "socially just", correcting, or at least not aggravating inequalities of wealth and power in society.

Although the viewpoints taken in this collection of essays are not new, they seem to require periodic restatement in a country in which deep-rooted emotional and psychological problems inhibit the development of any consistent or rational policy regarding land. Ann L. Strong, the most widely known of the authors, is surely right in her contention that in the United States there has always been an ultimate public interest in the care and custody of land. No less a libertarian than Thomas Jefferson made this explicit. But the myth of absolute and uninhibited private ownership has dominated public policy even to the demonstrable detriment of most private land owners.

The principal value of this collection of essays is that it reiterates the terms of the debate and keeps the discussion going at a time when the prospects for the reforms that most of the authors advocate appear unpromising. I agree with Ann Strong in the title of her essay, "Land As A Public Good: An Idea Whose Time Has Come Again", but I recall that the idea of a national land ethic implemented through public law has come among Americans before, and has not been received. I believe that the force of circumstances will inevitably cause the concept of land as a public good to be generally accepted, but I am not yet persuaded that that time is now. □

Lynton K. Caldwell is Bentley Professor of Political Science at Indiana University, and participated in drafting the United States National Land Use Policy Act of 1970.

A waste of time for everybody?

Susanna Millar

Animal Play Behavior. By Robert Fagen. Pp.684. Hbk ISBN 0-19-502760-4; pbk ISBN 0-19-502761-2. (Oxford University Press: 1981.) Hbk £21, \$29.95; pbk £10.50.

THE cover notes and preface to this book declare that Fagen has broken new ground by adopting an evolutionary approach to play, and that he has solved an important biological paradox. This is a somewhat inflated claim. With the single exception of behaviourism, now long outmoded, there is no theory of play, and no developmental theory, that has not assumed an evolutionary context or has operated in a biological vacuum since Spencer first tried to account for play, and Darwin started child watching. The introduction explains that only analyses based on sociobiology and behavioural ecology count. Fagen anticipates that this will cause offence. It is not at all clear why, except that the aggressive, polemical style seems to invite it. Muted hope, that the proposals will justify the claims made for them, is a much more likely response.

Fagen does, nonetheless, make a worthwhile contribution; more to method than to theory, but potentially all the more useful for that. He proposes to apply formal modelling techniques to play, using assumptions based on current genetic and evolutionary ideas. Formal mathematical and computer models, designed to simulate behaviours of complex systems, can be used to investigate the properties of the systems and are widely used in research in biology, genetics and psychology. Fagen's examples of possible model fitting with questions related to play, given mainly in appendices, are much the most interesting parts of the book and provide clear pointers to what could be done.

The main problem about play, according to Fagen, is that animals geared to survival apparently "waste time" by playing. Actually, this is more a terminological quibble than a biological paradox. The "useful/useless" dichotomy depends on the system of classifying behaviour by the outcome, goal or end-state which is obvious to the observer. It is arbitrary and, therefore, provides no reason against analyses of play in terms of survival value; or against applying the theory of frequency-dependent natural selection of genetically based variations to play behaviours. But there is also a genuine difference between play and behaviours such as sex and aggression, which here gets rather lost. This is the paradoxical relation between play activities and the contexts

(outcomes) in which they occur. It poses no problem for biological principles, and the "useful/useless" distinction is irrelevant to it. But the relation between activity and context does differentiate play from other behaviours, and it deserves rather more attention than it is accorded by Fagen.

By far the largest part of the book is devoted to the assumptions that Fagen has selected to explain play. These are given as chapter and section headings for his arguments. Since we do not as yet have the results of independent tests, these arguments carry no more weight than any other explanation based on observational and experimental studies. Fagen argues that play is an innate general tendency to prepare for later versatility, with possible connections to art and science. This view is not new. More importantly, it is not implied by evolutionary principles. The fact that it is, of course, unlikely that play behaviours would occur if they did not help with "fitness" and species survival in various ways does not by any means logically imply that all types of play must have the same function, or depend on a single mechanism. As Fagen is aware, such issues require testing rather than rhetoric. The eventual results of the modelling studies he proposes should certainly be of interest.

The book is meant to educate everybody. There is a lively chapter of descriptions, mainly of play-fighting, and one giving a brief account of some biological principles. Non-specialists may find it difficult to follow some of the arguments as they dart between assumption, assertion, findings and interpretation. In addition there are overstatements and inaccuracies. For instance, Fagen shows that cost-benefit tables can, in principle, be set up for play with various criteria of "fitness". But there are methodological difficulties in outcome studies that are passed over rather too lightly. The relaxed "play-face", which is a signal for social play in many species, is sometimes observed in solitary object and locomotor play. Fagen treats this as important evidence for the unity of play; but the observations are quite insufficient to decide whether the "play-face" in that case acts potentially even as a sign for others, let alone that it has the same signalling function as in social play. Piaget's theory is outdated; but to state that it considers play merely an epiphenomenon is rather a howler.

In all, there is an air of haste and of conducting a jihad which does a disservice to scholarship and to the subject. Nevertheless, students who already know something about the subject should find the book stimulating. □

Robert S. Desowitz's *New Guinea Tapeworms and Jewish Grandmothers* (reviewed in *Nature* 296, 514; 1982) will be published next week in the United Kingdom by W. W. Norton, price £9.25.

Susanna Millar is a Research Officer in the Department of Experimental Psychology at the University of Oxford.